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VOL. 19, SEC. C. FEBRUARY, 1941 NUMBER 2

ÉTUDES SUR LES HYBRIDES DE CISTES

IV. CORRÉLATION DES CARACTÈRES DU *C. SALVIIFOLIUS* L.[1]

PAR PIERRE DANSEREAU[2]

Sommaire

Les variétés du *Cistus salviifolius* proposées jusqu'ici par les auteurs ne paraissent pas valides, car l'examen d'un grand nombre d'individus révèle le manque de cohésion des caractères invoqués pour définir ces variétés. Ceci est confirmé par un examen statistique méthodique. Il est démontré, en outre, qu'il n'existe pas de liaisons très nettes des caractères, même considérés deux par deux.

Validité des variétés décrites

On a décrit un grand nombre de variétés du *Cistus salviifolius* L. Dans une monographie du genre (2), l'auteur n'a cru devoir retenir aucune de ces entités, car il lui a paru que la plupart des individus ne correspondaient pas aux descriptions des diverses formes en question. Ainsi, selon Grosser (5), la longueur des pédoncules est censée varier de 2 à 3 cm., puis de 4 à 8 cm.; la longueur des sépales de 7 à 12 mm., puis de 18 à 20 mm. Or, il ne manque pas de plantes ayant des pédoncules de 3 à 4 cm., et des sépales de 12 à 18 mm. Deux autres caractères sont invoqués par Grosser: le nombre des fleurs (un, deux, trois)*, et la présence d'inflorescences latérales. Le premier, de toute nécessité, présente des alternatives sans terme de passage. Pour le second, la plupart des plantes où on le rencontre portent aussi les autres types d'inflorescence, de sorte qu'il n'est pas, lui non plus, absolu.

Chacun des caractères invoqués par Grosser présente donc une variation insensible et n'est susceptible d'aucune répartition en catégories nettes. La seule défense qu'on pourrait faire de ces micromorphes serait leur valeur pratique, leur commodité. Or, il n'en est rien, puisque ces caractères sont rarement liés entre eux, contrairement à ce qu'impliquenent les descriptions jordaniennes de Timbal-Lagrave (9), de Rouy et Foucaud (8), et même celles, plus compréhensives, de Willkomm (10), et de Grosser (5).

Aucun agencement de caractères à l'intérieur du *C. salviifolius ne présente* la même solidité et la même cohésion que ceux qui servent à définir les var. *symphytifolius* et *leucophyllus* du *C. symphytifolius* ou les var. *minor* et *major* du *C. populifolius*, pour prendre des exemples dans le même genre, et où sont pourtant mis en cause des caractères quantitatifs.

[1] *Manuscrit original reçu le 3 août 1940 et sous forme revisée le 14 novembre 1940.*

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* *On rencontre, exceptionnellement, des individus à quatre ou cinq fleurs (P, Fig. 1).*

Que la répartition de facteurs quantitatifs soit discontinue dans ces cas et continue chez le *C. salviifolius* peut être l'effet de ségrégations géographiques et d'autres facteurs étrangers au patrimoine héréditaire des espèces. Mais, il n'en demeure pas moins que le résultat atteint nous importe plus que les hypothétiques virtualités de chaque espèce, et que les critères des micromorphes ne se distinguent de ceux de l'espèce que par l'ordre de grandeur: netteté des caractères, corrélation et fréquence.

Test quantitatif

Après une étude détaillée de plusieurs centaines d'échantillons d'herbier provenant de l'aire entière de l'espèce et de très nombreuses herborisations dans la région méditerranéenne française et italienne, l'auteur a cru pouvoir composer une description complète, de cette espèce polymorphe (2).

Reprenant les caractères variables, il a paru intéressant à l'auteur d'en faire un tableau statistique. C'est par ce moyen, semble-t-il, qu'on peut dessiner le profil biologique d'une entité taxonomique. Qui dit profil, dit contour, limite ou frontière: région de contact avec les entités affines. La statistique permet d'établir la densité d'un caractère et sa résistance aux apports extérieurs dans le cas d'espèces pouvant s'hybrider comme les *Cistus*. Et surtout, la statistique est un test de la validité des entités taxonomiques. Toute révision de groupe, et à fortiori, toute monographie, impliquent une statistique puisqu'elles supposent l'examen d'un matériel plus abondant que celui qui a servi aux descriptions antérieures.

Le test quantitatif envisage l'étendue de la variation des caractères mesurables et le calcul des nombres relatifs d'individus affectés par chaque phase de la variation. De cette tabulation ressortent les traits caractéristiques d'une espèce dans son ensemble, qui ne sont pas observables sur chaque spécimen. Plus un groupe sera homogène et plus grand sera le nombre des individus rencontrés au hasard qui répéteront exactement la formule trouvée pour l'ensemble du groupe. Celle-ci sera extrêmement différente selon qu'on l'appliquera à des espèces fermées (monotypes comme les *Sanguinaria canadensis*, *Tamus communis*, *Dalibarda repens*, *Crambe maritima*), ou à des espèces ouvertes (*Rosa blanda*, *Euphrasia Rostkoviana*, *Rubus orientalis*, *Alchemilla vulgaris*, *Cistus salviifolius*). Autrement dit, l'analyse quantitative ne pourra que réfléter le mode de variation propre à un groupe taxonomique donné et qui est ordinairement celui de sa genèse.

En effet, quoi que nous puissions savoir des influences qui agissent sur une espèce donnée, ou même des constituants qui l'ont formée dans le passé, en ce moment actuel de son devenir, il nous faut bien nous la représenter comme la somme des caractères présents chez le plus grand nombre possible des individus qui la concrétisent. Au point de vue biologique, c'est la moyenne, la somme des tendances, qui est le type.

De sorte que, pour chaque espèce, on peut dessiner un profil qui la situe dans le genre et qui exprime le travail de différenciation qui s'y opère dans

son état actuel. Les caractères importants sont les mieux différenciés, les plus nettement spécialisés puisqu'ils servent par excellence à isoler l'espèce, à lui conférer une identité d'autant plus nette qu'elle sera seule à les posséder. Lorsque ces caractères (et le plus grand nombre possible) ont atteint une certaine stabilité (i.e. une présence à un haut pourcentage chez les individus), l'espèce est bien caractérisée. Peut-être approche-t-elle du terme de son évolution et vieillit-elle? Il en va de même à l'intérieur de l'espèce pour les variétés et les formes.

Le test quantitatif, donc, sera particulièrement utile si on l'applique à l'analyse d'espèces encore dynamiques, mal fixées, et plus ou moins insaisissables par les moyens classiques de la taxonomie. Il permettra de déceler les zones de plus grande densité qui existent dans un groupe et de connaître la relation entre l'écart constitué par les extrêmes et le point de concentration du maximum d'individus. Le test quantitatif dégage l'état actuel d'un groupe taxonomique, et sa position précise par rapport aux groupes voisins.

Test qualitatif

Le test qualitatif, d'autre part, recherche des caractères exclusifs ou discontinus de par leur nature même. Ainsi, le nombre de fleurs, un, deux, trois, etc., ne suppose aucun intermédiaire. D'autres cependant, le sont en fait et ceux-là sont intéressants à connaître par la statistique, puisque, à priori, ils appartiennent à des séries qui peuvent comporter des formes de passage. Ainsi les *Solidago canadensis* et *S. lepida* ont des capitules respectivement de 2 à 2.8 mm. et de 3 à 8 mm. de haut; les *Circaea alpina* et *C. latifolia* ont des feuilles respectivement de 3 à 5 cm. et de 5 à 10 cm. de long (7).

Or, la discontinuité des caractères variables est souvent relative; c'est dire qu'elle ne ressort que d'un grand nombre d'observations. Ainsi, le *Populus Tremula* a des feuilles à peu près aussi larges que longues, souvent plus larges que longues, parfois plus longues que larges, tandis que le *P. Tremula* var. *Freyni* a des feuilles toujours plus longues que larges (4). Les deux groupes ont donc une zone de contact, mais cette zone n'implique qu'une faible partie des individus d'un type hétérogène, et n'infirme en rien la solidité de la variété *Freyni* qui apparaît fort homogène.

La discontinuité peut être observée aussi bien dans une corrélation ou un manque de corrélation entre deux séries de caractères qu'à l'intérieur d'une série d'allélomorphes. Ainsi, les *Oenothera* à étamines égales sont de grandes plantes; les *Cistus* à fleur rouge ont tous un long style, mais un seul *Cistus* à fleur blanche possède ce caractère.

La biométrie permet donc d'établir le coefficient d'instabilité d'un groupe. Ceci peut porter sur le grand nombre des individus et permettre de connaître les extrêmes et la catégorie ou les catégories où se sont formées des concentrations. Appliquée, d'autre part, à l'ontogenèse, et non au seul produit terminal qui sert le plus souvent de base à la définition des groupes, la statistique biométrique permettra une appréciation qualitative des critères spécifiques

d'une très grande valeur. Jentys-Szaferowa (6) a pu ainsi rendre compte du devenir et de la fixation de la forme des feuilles chez les diverses espèces que Linné englobait dans son *Betula alba*: *B. verrucosa*, *B. oycoviensis*, *B. carpatica*, *B. pubescens*. De telles études sont extrêmement précieuses pour la taxonomie, puisqu'elles permettent de toucher et de définir objectivement le processus de la différenciation non seulement dans ses effets mais peut-être aussi dans son action.

En envisageant les problèmes taxonomiques sous cet angle, on pourra se demander sur quels caractères communs il convient de baser les groupes et en vertu de quels écarts, ou de quelles solutions de continuité, il est permis de tracer des frontières. La discontinuité n'est pas forcément le critère final. On peut facilement imaginer une espèce qui ne contienne absolument aucun caractère qui lui soit exclusif, mais une combinaison de caractères exclusive. C'est le cas où le test quantitatif serrera la vérité de plus près que le test qualitatif, plus conventionnel.

Dans la pratique, cependant, les caractères exclusifs sont sans doute les plus intéressants dans un test qualitatif puisqu'ils résultent soit d'une survivance unique ou d'une différenciation très avancée. La fidélité, d'autre part, de certain caractère à tel ou tels autres suppose aussi une fixation, un point terminal dans une voie de différenciation.

Dans le cas qui nous intéresse ici, on peut se demander quel facteur de chaque série est le plus typiquement "*salviifolius*", et ceci indépendamment de sa répartition numérique. C'est-à-dire qu'on peut classer les caractères d'après leur exclusivité à l'espèce en question. Le *C. salviifolius* tient déjà sa consistance de quelques caractères invariables et indissociés. L'un quelconque d'un certain nombre d'autres caractères peut être assimilé à ceux-ci dans la mesure où il partage cette exclusivité, en autant aussi qu'on le trouve associé aux précédents. Le manque d'abondance numérique, s'il y a lieu, ne signifie alors qu'un affaiblissement de la cohésion du complexe "*salviifolius*", ou peut indiquer, au contraire, que le stade actuel est subterminal et que cette cohésion est encore en voie de réalisation, l'espèce étant encore jeune et non stabilisée, mal dégagée encore des influences qui l'ont formée.

Si le pourcentage de coïncidence est très élevé, il ne subsiste alors guère de doute à cet égard et les caractères alternatifs de la même série que le caractère en question n'apparaissent plus que comme des liens, des canaux, si l'on veut, rattachant l'espèce à d'autres espèces (ou rejoignant le phylum primitif du genre?).

Le test qualitatif relève donc la valeur des discontinuités et permet d'apprécier le degré de la différenciation dans le groupe de caractères où elle est encore active. Il se trouvera parfois que cette différenciation sera peu avancée encore pour certains caractères, dans des groupes taxonomiques bien isolés mais dont l'isolement peut être dû surtout à des causes extérieures et non à la structure interne de l'espèce*.

** Ce serait le cas, par exemple, du Sanguinaria canadensis, espèce monotype où le nombre des pétales, la forme des feuilles, la longueur des hampes florales, etc. sont mal fixés.*

Degré de présence des caractères étudiés chez le *C. salviifolius*

Les spécimens étudiés ici ont presque tous été récoltés depuis moins d'un siècle, espace de temps sans doute assez insignifiant dans la vie d'une espèce aussi largement répandue*.

Les caractères absolus donc sont laissés de côté, puisqu'ils ne manquent dans aucun échantillon étudié et qu'ils figureraient dans les statistiques comme présents à 100%. Ils sont présumés avoir atteint une différenciation définitive et je les appellerai ici caractères primaires: (i) fleur blanche, (ii) cinq sépales, (iii) feuille pétiolée, (iv) style court, (v) cinq loges à l'ovaire, (vi) feuille de moins de 50 mm. de long. Ces six caractères ne se retrouvent jamais ensemble dans une autre espèce: le premier exclut tout le sous-genre *Erythrocistus*; le second exclut les sections *Ladanium* et *Halimioides*; le troisième exclut les *C. monspeliensis* et *C. hirsutus;* le quatrième exclut le *C. varius*, le cinquième le *C. ladaniferus*, et le sixième le *C. populifolius*. Ces caractères primaires suffisent donc à définir le *C. salviifolius*, et toute variation qui ne les atteint pas est insuffisante pour faire sortir l'individu affecté de son orbite spécifique.

Les caractères variables ou secondaires, sont représentés dans la Fig. 1. La procédure suivie a été la même que pour le *C. monspeliensis* (1). Chaque spécimen a été analysé pour cinq séries de caractères représentés par des lettres, de sorte qu'on a pu attribuer à chacun une formule (*CEILN*, *ADHKN*, etc.). La somme totale de ces formules se trouvera donc exprimer à la fois la fréquence de chaque caractère individuel, leur corrélation entre eux et la distribution géographique des gènes qui les déterminent (Fig. 2).

Trois séries de caractères se rapportent aux feuilles (Fig. 1). D'abord leur dimension: feuilles de plus de 45 mm. de long (*A*), de 25 à 45 mm. (*B*), et de moins de 25 mm. (*C*). On voit (Fig. 2) que les plus petites feuilles (*C*) sont les plus fréquentes, que les grandes feuilles (*A*) sont rares, au plus, et exceptionnellement, 21% en Algérie. Il est à remarquer qu'il ne s'agit ici que des feuilles des rameaux fertiles et non des rameaux stériles et des rejets, dont la forme et les dimensions comme chez bien d'autres espèces (*Platanus*, *Populus*, etc.) varient énormément (4). On peut donc constater la dominance de la microphyllie et supposer que la recombinaison la favorise, étant donnée son abondance actuelle en individus. On peut alors envisager qu'elle résulte d'une meilleure adaptation aux conditions écologiques de l'espèce, ou qu'elle est liée à quelque facteur interne. Ceci est appuyé par le fait que le pourcentage des mésophylles est environ sept fois plus élevé que celui des macrophylles.

Quant à la forme des feuilles, (Fig. 1) elle peut-être: cordée (*D*), ovale (*E*), orbiculaire (*F*), ou allongée (*G*). Les feuilles ovales sont de beaucoup les plus fréquentes étant, en moyenne, et dans presque chaque secteur, plus de deux fois plus nombreuses que chacune des trois autres catégories.

** Il y a une réserve à faire à ce sujet: le milieu où croissent les C. monspeliensis et salviifolius a encore changé depuis un siècle et a favorisé la dispersion du premier (1, 3).*

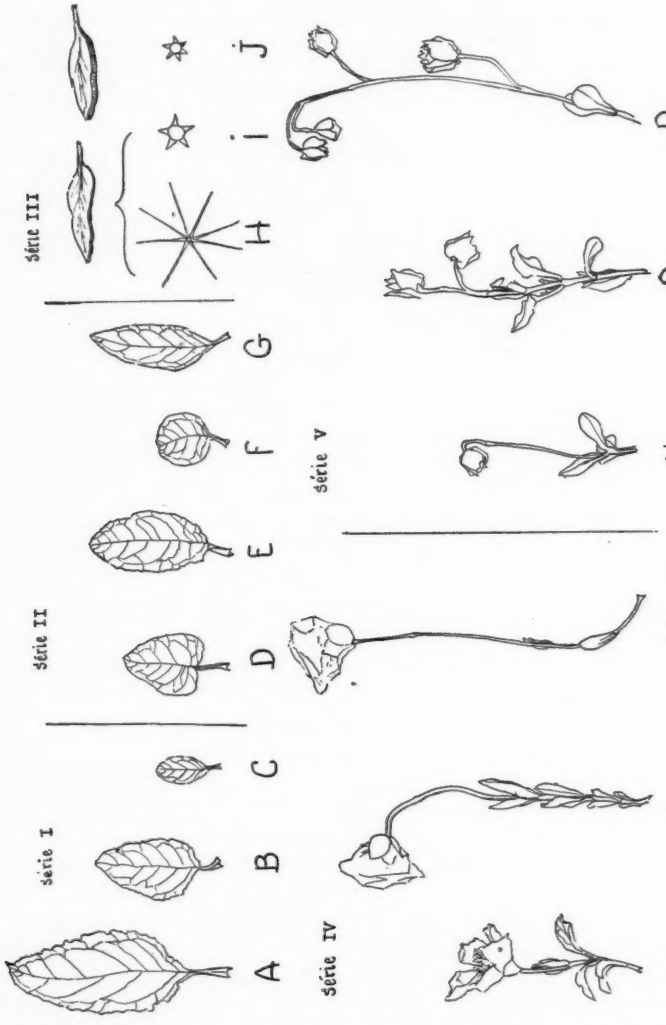

FIG. 1. *Caractères du Cistus salviifolius. Série I. Longueur de la feuille: (A) 45 mm. et plus, (B) 25 à 45 mm., (C) moins de 25 mm. Série II. Contour de la feuille: (D) cordée, (E) ovale, (F) orbiculaire, (G) allongée. Série III. Indument et texture de la feuille: (H) texture membraneuse et poils étoilés grêles, (I) texture membraneuse et poils étoilés raides, (J) texture spongieuse et poils étoilés raides. Série IV. Longueur de l'inflorescence: (K) 6 à 10 cm., (L) 4 à 6 cm., (M) moins de 4 cm. Série V. Nombre de fleurs: (N) une, (O) deux ou trois, (P) plus de trois.*

L'indument des feuilles peut se composer de poils étoilés à branches longues qui donnent à la feuille un aspect tomenteux (*H*), ou de poils étoilés à branches courtes et rigides formant surface verruqueuse (*I*); dans ces deux cas, la texture de la feuille est nettement membraneuse; dans un autre cas (*J*) la texture de la feuille est spongieuse et sa surface nettement verruqueuse. Cette dernière forme est assez peu fréquente. La répartition des deux premières, (*H* et *I*) est moins nette, les proportions indiquées ici ne pouvant être prises trop littéralement; car si la moyenne des (*H*) est de 9% plus élevée que la moyenne des (*I*), par contre dans six secteurs sur neuf, ce sont les (*I*) qui

l'emportent. Il faut admettre, en outre, que parfois les feuilles d'un spécimen présentaient un caractère intermédiaire qui rendait le classement difficile sinon un peu arbitraire.

		Lomb. Pié. Fr. W. Suisse	Fr. Médit. Italie N.	Esp. et Port.	Italie C. et S. et Sicile	Baléares Corse et Sard.	Grèce, Turq. et Asie Min.	Tunisie	Algérie	Maroc	Moyenne
Série I	A	4	8	4	4	3	4	5	21	5	6
	B	35	46	33	34	14	43	67	43	58	42
	C	61	46	63	62	83	53	28	36	37	52
Série II	D	6	8	15	6	0	10	5	4	9	8
	E	62	50	52	49	47	41	79	45	57	51
	F	13	21	19	27	39	5	3	15	16	19
	G	19	21	14	18	4	44	13	36	18	22
Série III	H	52	41	32	33	28	32	69	31	39	38
	I	42	38	55	54	61	62	20	48	49	49
	J	6	21	13	13	11	6	11	21	12	13
Série IV	K	25	32	16	13	11	11	9	17	23	23
	L	57	41	36	41	25	45	63	54	40	43
	M	18	27	48	46	64	44	28	29	37	34
Série V	N	78	65	44	54	80	40	75	54	70	65
	O	21	33	45	36	6	56	25	46	27	31
	P	1	2	11	10	14	5	0	0	3	4

FIG. 2. *Répartition des caractères du C. salviifolius exprimée en pourcentage du nombre total des individus examinés et pour chaque région. Les lettres (de A à P) correspondent aux caractères illustrés dans la Fig. 1.*

Les deux dernières séries d'observations portent sur l'inflorescence. La longueur peut être de 6 à 10 cm. (*K*), de 4 à 6 cm. (*L*), ou de moins de 4 cm. (*M*). La répartition se présente ici sous la forme d'une courbe régulière, le type moyen étant le plus nombreux et les extrêmes atteignant des proportions élevées. La série est donc quantitativement continue et on peut croire que le jeu des facteurs y est très libre.

Quant au nombre des fleurs, le nombre un (*N*), dépasse du double en moyenne, les nombres deux à trois (*O*), et les individus multiflores (*P*), rarissimes, manquent même totalement à certains secteurs.

La formule *CEILN* correspond à la moyenne la plus élevée pour chaque série de caractères. Or, les caractères *CEIN* sont, dans les séries étudiées, les plus nettement exclusifs au *C. salviifolius*; la longueur d'inflorescence de 40 à 60 mm. (*L*) se trouvant chez toutes les espèces sauf les *C. crispus* et *parviflorus*, notamment chez les espèces affines: *C. hirsutus*, *populifolius*, *monspeliensis*.

Les feuilles plus courtes que 25 mm. (*C*) constituent un caractère exclusif au *C. salviifolius* dans la section *Ledonia*, mais se retrouvent à un haut pourcentage chez le *C. monspeliensis* dans la section *Stephanocarpus* (1). Il faut donc considérer ce facteur comme commun aux deux espèces (puisqu'on le retrouve chez la seconde aux Iles Canaries où manque le *C. salviifolius*). Cette forme ne se retrouve guère dans les autres espèces du genre appartenant à des sections plus éloignées.

La texture verruqueuse des feuilles (*I*) ne reparaît nettement que chez le *C. heterophyllus*, espèce sans liens évidents avec le *C. salviifolius*.

Les inflorescences uniflores (*N*) n'existent que chez les *C. heterophyllus*, *salviifolius*, *ladaniferus*. Malgré l'existence d'hybrides entre ces deux dernières espèces, on ne peut parler d'affinités étroites, vu le nombre et l'importance des caractères qui les séparent, et singulièrement ceux de la fleur et de l'inflorescence.

En résumé donc: le *C. salviifolius* se détache nettement de tous ses congénères par l'association constante de six caractères primaires, accompagnée de la présence fréquente de quatre caractères secondaires (*CEIN*, Fig. 1), plus ou moins exclusifs mais caractéristiques du fait de leur association avec les précédents. Voilà définie la cohésion de l'espèce, sa solidité, sa résistance à l'extérieur*.

L'étude des proportions et des liaisons établies entre les caractères secondaires et leurs alternants nous permettra de nous représenter le dynamisme interne de l'espèce et ce qui lui reste de voies de communication avec d'autres espèces.

Mécanisme de la répartition des caractères chez le *C. salviifolius*

Les écarts à partir des caractères *CEILN* qui sont les plus fréquents, sont dus à plusieurs causes:

(1) Hétérozygotie de certains allélomorphes;
(2) Changements écologiques et plasticité relative de l'espèce à cette égard;
(3) Localisation géographique de certains gènes;
(4) Introgression de certains facteurs par hybridation avec d'autres espèces, soient les *C. monspeliensis*, *hirsutus*, *populifolius*.

* *Le genre Cistus est peut-être plus qu'un autre pauvre en gènes, et ses espèces, pour être nettes, n'en résultent pas moins d'un petit nombre de facteurs associés différemment* (*3*).

Il est clair que ces causes peuvent agir conjointement; que la quatrième, par exemple, engendre la première, etc. Quelques cas, cependant, ressortissent à l'une plutôt qu'aux autres.

On ne peut que considérer l'espèce entière comme hétérozygote pour les couples $B-C$, $H-I$, et même $N-O$ (Fig. 2). Ces caractères s'opposent très nettement, et leur présence à un pourcentage très élevé dans chaque secteur, laisse supposer qu'il doit se trouver peu d'individus homozygotes pour l'un ou l'autre des allélomorphes.

Les caractères quantitatifs (longueur des feuilles (A, B, C) et des inflorescences (K, L, M)) subissent forcément l'influence du milieu; on ne sait malheureusement pas si la répartition des plantes à grandes feuilles et à longues inflorescences coïncide toujours avec l'habitat sylvestre et mésophile. La culture, d'autre part, a montré l'influence du sol sur la forme et les dimensions des feuilles. Le diamètre de la fleur varie énormément avec l'exposition et la température (q.v.).

Par contre, la répartition de l'indument des feuilles subit des localisations assez intéressantes (Fig. 2). Les feuilles blanchâtres, veloutées, feutrées (H) se rencontrent surtout au nord de l'aire: région extra-méditerranéenne, nord de l'Italie, sud de la France, Maroc (où la plante atteint une certaine altitude); tandis que la feuille affecte davantage la surface verruqueuse (I) dans les régions où l'habitat xérophytique est plus général (Italie moyenne et méridionale, Espagne, Asie Mineure, Grèce, etc.).

D'autres caractères sont assez nettement hybridogènes. Les feuilles cordées à la base se rencontrent fréquemment en Espagne, dans la France méditerranéenne du sud-ouest, et au Maroc, où l'hybridation avec le *C. populifolius* n'est pas rare. Deux autres caractères sont attribuables à une hybridation plus ou moins ancienne avec le *C. monspeliensis:* la forme allongée des feuilles (G) et un nombre de fleurs supérieur à trois (P). Or, leur localisation géographique respective ne coïncide aucunement. Les individus polyflores (P) sont les plus nombreux dans les îles de la Méditerranée, en Italie et en Espagne; ceux à feuilles allongées (G) en Grèce, en Asie Mineure et en Algérie. Faut-il admettre qu'un même croisement est suivi de recombinaisons différentes à cause de la sélection du milieu?*

Corrélation des caractères du *C. salviifolius*

Le nombre des plantes examinées est 687: 324 combinaisons des 16 caractères étaient possibles. Seulement 151 se sont réalisées. Les caractères C, E, I, L, N, se sont présentés individuellement le plus souvent. Or, la combinaison de cinq caractères la plus fréquente a été $CEIMN$ (41 fois, soit un peu moins de 6%), tandis que la combinaison $CEILN$ est deux fois moins fréquente.

Il devient donc intéressant de rechercher si ces caractères sont de quelque manière liés entre eux, par deux, par trois, par quatre, ou par cinq. On peut

** On peut voir là une tendance, mais une tendance seulement, à la formation d'une variété géographique nouvelle.*

considérer les cinq caractères primaires (voir plus haut) comme liés ensemble à 100% et chacun des caractères "secondaires" comme lié à 100% avec eux; tandis que les primaires ne sont liés aux secondaires que dans une proportion toujours très inférieure à 100, mais d'autant plus significative qu'elle s'en rapproche. Un caractère (comme *N*) qui dépasse 50% de fréquence est plus étroitement assimilable aux caractères primaires que les autres et singulièrement que ses alternants (*O* et *P*). De même, un groupe de caractères, atteignant ensemble (par deux, trois, quatre, cinq) un haut pourcentage du total des individus constituerait à l'intérieur de l'espèce un aggrégat homogène méritant un rang taxonomique, puisqu'il résulterait d'une spécialisation définissable. Ce rang taxonomique serait naturellement plus élevé s'il s'agissait de cinq que de quatre, de quatre que de trois caractères, etc.

Or, en ce qui concerne les combinaisons de cinq caractères, la corrélation est très faible *CEIMN* = 5.96, *CEHLN* = 4.07, *BEILN* = 3.34%, etc. Ces chiffres marquent évidemment une absence presque totale de cohésion des caractères considérés par cinq.

Les associations de quatre caractères ne sont guère plus prometteuses. *CEIN* et *CIMN* nous donnent 10% du total des individus analysés, *CELN*, 9, *CEMN*, 8.3, *EIMN*, 8%. Ces chiffres sont d'autant moins significatifs que ces cinq combinaisons ont entre elles, prises deux par deux, au moins deux et souvent trois caractères communs.

	I	II	III	IV	V
I	2	4	4	4	4
II	4	2	6	6	5
III	4	6	2	7	5
IV	4	6	7	2	5
V	4	5	5	5	2

FIG. 3. *Tableau des corrélations. Les chiffres romains indiquent les numéros des séries de caractères alternants représentées dans la Fig. 1. Les chiffres arabes se rapportent à la figure ou la corrélation entre les séries prises deux par deux est relevée.*

Sans nous arrêter aux combinaisons de trois facteurs, voyons le tableau de tous les facteurs deux par deux. La Fig. 3 donne le chiffre du tableau où sont relevés les pourcentages d'association de chaque série de caractères (Fig. 1) par rapport à chacune des quatre autres séries. Les figures 4 à 7 donnent donc un tableau complet des associations de caractères. Deux considérations s'imposent ici. Premièrement, existe-til deux caractères liés d'une façon exclusive ou presque? Prenons, par exemple l'inflorescence uniflore (*N*) présente chez 65% du total des individus (en moyenne) et la feuille ovale (*E*), présente chez 51% des individus. A priori, la liaison ne

peut être totale, puisqu'elle ne saurait dépasser 51%. Autrement dit, tous les *E* pourraient être liés à des *N*, mais tous les *N* ne sauraient être liés à des *E*, le nombre total de ceux-ci étant inférieur à 65%. En fait, la Fig. 5 nous montre

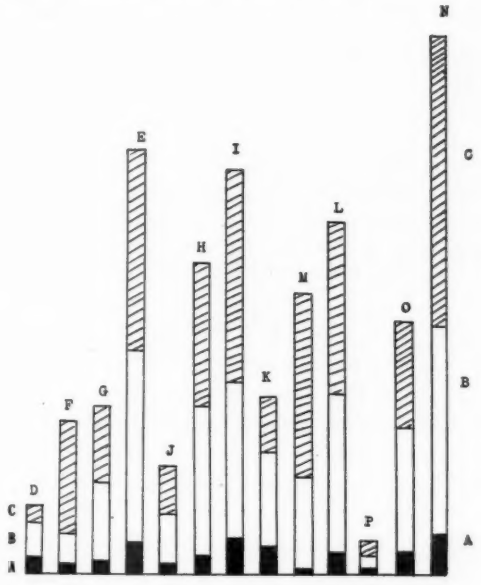

FIG. 4. *Corrélation des facteurs (A, B, C) de la première série avec ceux des quatre autres séries.*

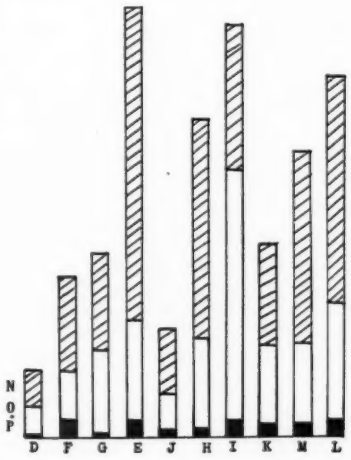

FIG. 5. *Corrélation des facteurs (N, O, P) de la cinquième série avec ceux des séries II, III, et IV.*

que la liaison *EN* se rencontre chez 37% du nombre total des individus. Ce chiffre correspond à environ 72% des *E* et 57% des *N*. Le reste des *E* (28%) et des *N* (43%) se réparti à peu de chose près proportionnellement au pourcentage moyen du caractère (*D*, *F*, *G*, et *O*, *P*) auquel on les trouve associés.

La Fig. 2 nous avait déjà démontré que nous avions affaire (surtout dans les séries I, II, III, et V) à deux caractères plus ou moins également répartis et à une troisième alternative beaucoup moins fréquente.

Ces derniers, sur les graphiques présentés ici, sont placés en bas. L'on constate qu'ils occupent généralement une longueur proportionnelle à la colonne où ils se trouvent. Les caractères atypiques par excellence donc ne sont ni liés entre eux ni liés à aucun autre caractère, du moins pas dans une proportion significative.

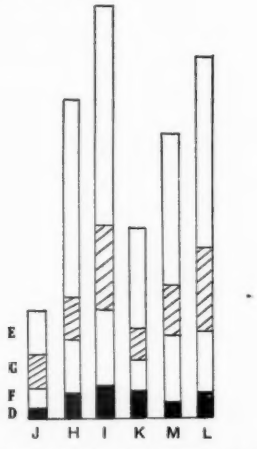

FIG. 6. *Corrélation des facteurs (D, E, F, G) de la série II avec ceux des séries III et IV.*

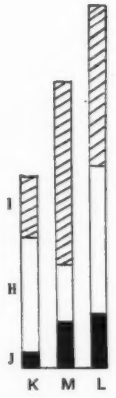

FIG. 7. *Corrélation des facteurs des séries III et IV.*

La seconde recherche, en l'absence de liaisons absolues, portera sur ce qu'on peut appeler les préférences. On peut classer ainsi une association atteignant un haut pourcentage du total ou dépassant de beaucoup les pourcentages atteints par toutes les autres combinaisons de deux facteurs des deux mêmes séries, ou encore toute association de deux caractères non proportionelle à leurs pourcentages respectifs.

Dans le premier cas, on arrive à définir plus exactement le type que par les seuls relevés de la Fig. 2; et dans le second cas, on peut discerner des tendances sinon un commencement de ségrégation.

Le *C. salviifolius* sera donc typique quand il possédera les caractères: (i) feuilles petites (*C*) ou moyennes (*B*), (ii) ovales (*E*), (iii) blanchâtres (*H*) ou verruqueues (*I*), (iv) l'inflorescence moyenne (*L*) ou longue (*K*), (v) uniflore (*N*). La seule liaison qui mérite d'être appelée typique est la liaison *EN*.

Les autres caractères ne manifestent que des tendances: (i) les feuilles grandes (*A*), (ii) cordées (*D*), (iii) subcharnues (*J*), (iv) les inflorescences courtes (*M*), (v) biflores (*O*) ou multiflores (*P*). Pour les caractères des troisième et cinquième séries, deux tendances opposées se manifestent: inflorescence uniflore, feuilles blanchâtres (*HN* = 25% du total), et inflorescences à deux ou trois fleurs, feuilles verruqueuses (*IO* = 30% du total). Le facteur inflorescence courte (*M*) semble, lui aussi, se lier plus que ses alternants (*L* et *K*) au facteur feuille grande (*A*) i.e., 3.6% du total, soit presque 50% des (*A*).

Aucune de ces tendances, si intéressantes soient-elles au point de vue biologique, ne mérite, à mon avis, d'être reconnue comme entité taxonomique distincte. Il est particulièrement à noter que les caractères si souvent invoqués de l'inflorescence plus ou moins longue (*K*, *L*, *M*) et surtout biflore à multiflore (*O*, *P*) ne sont liés à aucun autre d'une façon constante.

Conclusions

Le *Cistus salviifolius* est une espèce très polymorphe comme le prouve la répartition numérique de plusieurs séries de caractères.

D'autre part, la répartition de ces caractères chez les individus prouve qu'ils ne manifestent entre eux qu'une cohésion négligeable: il devient à peu près impossible de décrire des micromorphes basés sur plus d'un caractère.

L'hybridation intraspécifique est donc supposée intense, d'autant plus que les Cistes sont généralement autostériles. Cette variation continue est accentuée par l'action du milieu.

L'hybridation avec d'autres espèces (les *C. monspeliensis*, *hirsutus*, et *populifolius*, surtout) permet l'introduction, dans un complexe déjà chargé, de caractères nouveaux (*G*, *P*) ou l'accentuation de caractères déjà existants.

Le présent essai n'est pas une étude biométrique, car le matériel étudié est forcément hétérogène (provenant de toute l'aire de l'espèce) et des chiffres trop précis n'auraient que très peu de valeur. L'auteur a seulement voulu démontrer la grande liberté du jeu des facteurs. Ce cas, dans le genre *Cistus* est extrême; les autres espèces suivent des voies de différenciation identifiables, tandis que le *C. salviifolius* est indifférencié par rapport à plusieurs groupes de caractères.

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EFFECTS OF TALC AND PHYTOHORMONE TREATMENT ON THE ROOTING OF *DAHLIA* CUTTINGS[1]

By N. H. Grace[2]

Abstract

Groups of *Dahlia* cuttings, untreated, talc treated, and treated with talc containing various concentrations of naphthylbutyric acid, were propagated in sand in a greenhouse. All the untreated cuttings died; those treated with talc alone suffered only 4% mortality. Although phytohormone treatment increased the number of roots per rooted cutting, it increased the average mortality to 23%. There were no significant differences in the effects of the various concentrations of phytohormone. Reduction of mortality by talc treatment was the chief feature of the results.

It is well known that *Dahlia* varieties may be propagated by means of stem cuttings (6). The manufacturers of several phytohormone preparations recommend the use of their products, at definite concentrations, for the successful propagation of *Dahlia* by this means; similar recommendations occur in the literature (5). However, the value of phytohormone treatments in the case of this plant has been questioned, in conversation, by several commercial propagators. This communication reports the results of an experiment in which *Dahlia* cuttings were treated with naphthylbutyric acid in a series of concentrations, in a talc carrier.

Experimental

The experiment involved the use of a group of untreated cuttings, a group dusted with talc only, and groups receiving dust treatments with 1- and 2-γ-naphthylbutyric acid at concentrations of 250, 500, 1000, and 2000 p.p.m. (parts of chemical to a million parts of the talc mixture by weight) (2, 4). There were five randomized replicates of the foregoing six treatments, with five cuttings to the group, 150 cuttings thus being required. *Dahlia* (variety Sagamore Gold) cuttings were taken from the plants on April 12, 1940, approximately four weeks after the tubers had been planted. The groups of five cuttings were weighed, dusted, and planted immediately in a relatively coarse brown builders' sand (3). For the first week after planting the propagation frame was covered with a factory cotton screen. The frame was not provided with bottom heat. The temperature of the greenhouse approximated 65° F., though it frequently rose to 75° F. during the day.

Cuttings were removed about five weeks after planting and records were taken of the number of cuttings dead, rooted, the number and lengths of root, the fresh root weight, and the green weight of the living cuttings. The numbers and lengths of roots per rooted cutting and the mean root length were calculated. Data on the numbers of cuttings were subjected to the inverse sine transformation prior to statistical analysis (1).

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. N.R.C. 967.
[2] *Biochemist.*

Results

The chief feature of the results relates to the effects of talc treatment. All cuttings with no treatment died within one week of planting. In consequence, the analyses of variance of the responses of *Dahlia* cuttings were confined to the five groups receiving treatment with talc only and talc containing naphthylbutyric acid. The results of the analyses of variance are given in Table I. It is apparent that statistically significant treatment effects were shown only by the number of cuttings dead and the number of roots per rooted cutting. Mortality following naphthylbutyric acid treatment was 23% and after talc treatment alone, 4%; the number of roots per rooted cutting was 3.7 and 1.5, respectively. Whereas phytohormone treatment increased both the number of roots and mortality, there were no statistically significant differences attributable to varying concentrations. Rooting, averaged over the experiment, was 48%. Although the data on rooting of the cuttings were too variable to demonstrate significant treatment effects, it may be mentioned that the proportion of cuttings rooted was: 36% of those talc treated, 51% of all cuttings treated with phytohormone in talc, and 60% of those receiving the best phytohormone-talc treatment.

TABLE I

ANALYSIS OF VARIANCE OF RESPONSES OF *Dahlia* CUTTINGS TREATED WITH NAPHTHYLBUTYRIC ACIDS IN TALC

Source of variance	Degrees of freedom	Mean square						
		No. of cuttings		No. of roots per rooted cutting	Length of roots per rooted cutting	Mean root length	Fresh root weight	Final green weight of cuttings
		Rooted	Dead					
Replicates	4	606.0	656.5*	3.30	17936	214.5	197.0	26.06
Treatments	4	417.4	355.2	9.78	9518	221.2	315.5	4.66
Talc *vs.* others	1	978.4	1260.3*	19.18*	6691	388.1	531.8	7.84
Error	16	344.7	193.5	4.06	10158	240.6	249.5	31.84

* *Exceeds mean square error, 5% level of significance.*

These results demonstrate that talc treatment is markedly beneficial in reducing mortality of *Dahlia* cuttings. The addition of phytohormone results in a marked increase in the number of roots formed, but tends to increase mortality.

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SOME FACTORS AFFECTING THE VIRULENCE OF ARTIFICIAL INOCULUM OF *HELMINTHOSPORIUM SATIVUM* P. K. AND B. AND OF *FUSARIUM CULMORUM* (W. G. SM.) SACC.[1]

By L. E. Tyner[2]

Abstract

Investigations were conducted on the virulence of inoculum of *Helminthosporium sativum* P. K. and B. and of *Fusarium culmorum* (W. G. Sm,) Sacc. as affected by the size of the vessel in which the inoculum was increased, the amount of corn meal present in the medium, and the period of incubation. Inoculum of either pathogen containing 12% corn meal caused more disease on wheat seedlings than that with 5%. *H. sativum*, 14 days old, was more virulent than after 21, 28, or 35 days' incubation, but in the case of *F. culmorum*, there was no definite tendency with respect to the effect of age. The size of container was unimportant if desiccation was avoided.

Introduction

Methods of producing artificial epidemics of air-borne diseases are quite well standardized, because the importance of etiological conditions is appreciated. The method usually employed is to apply a determinate spore load, either dry or suspended in aqueous solution. In the case of soil-borne diseases, it is the practice to apply the inoculum either to the soil in which the plants are grown, or to the seed itself. When it is added to the soil, a carrier such as coarsely ground oat hulls or soil, with various organic admixtures, is essential. Too frequently the effect of these adjuncts is overlooked in the production of the inoculum, with the result that inconsistent data are secured.

Sallans (2), reviewed the methods used by others to inoculate wheat with *Helminthosporium sativum* P. K. and B. and reported his own results. As inocula he used spore suspensions and spores borne in an oat hull medium. Infection was found to decrease with increase in age of the oat hull inoculum and also with curtailment of amount applied. An adverse effect of the carbohydrates in the carrier was noted in the controls, therefore he recommended the use of seed soaked in a suspension of the pathogen for extensive experiments.

Inasmuch as work at this laboratory has proved that soil combined with a minimum of corn meal is a satisfactory medium in which to increase *Helminthosporium sativum* P. K. and B. and *Fusarium culmorum* (W. G. Sm.) Sacc. for root-rot studies, information concerning the effect on virulence of certain factors, including size of the vessel in which the inoculum was increased, the amount of corn meal present, and the age of the inoculum was important.

Materials and Methods

Black loam containing 20% water, was mixed with 5 or 12% corn meal by weight, as required. The media were steam sterilized in 150 and 600

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gm. quantities in 200 and 1000 ml. Erlenmeyer flasks, respectively. When cool, a number of these flasks were seeded with aqueous spore suspension of *H. sativum* or *F. culmorum* and incubated at room temperature. Corresponding flasks were seeded at subsequent intervals to provide inocula of required ages.

The spore suspensions were added aseptically in amounts of 0.5 and 0.3 ml. to the large and small flasks, respectively. The suspensions were made by adding 10 ml. of sterile distilled water to a standard size test tube containing *H. sativum* or *F. culmorum*, cultured on slants of potato dextrose agar for two weeks.

The tests for virulence were made under greenhouse conditions in 6-in. pots. In the first and second experiments there were two series, namely, one with steam sterilized soil, and another with unsterilized soil. The sterilized soil series was omitted in the third experiment. Twenty surface-disinfected, uniform, Marquis wheat seeds were planted in four replicates and the inocula applied at seed level. Thirty grams of inoculum was used in the first and second experiments and 20 gm. in the third.

Notes on height and dry weight of the plants and degree of infection were taken after 40 days. The values obtained were weighted in order to compensate for the differences in numbers of surviving plants. For dry weight data, the actual weight of surviving plants was divided by the number of plants surviving in corresponding control pots. Infection data were weighted by allowing a rating of 100% on plants not surviving, either by failure to emerge or by death during growth. The standard was the number of plants surviving in the controls.

Results

Experiment I

This experiment was carried out during January and February, 1940. Inoculum of *H. sativum* only was employed, at the rate of 30 gm. per pot. Equivalent amounts of unseeded soil corn meal mixture were used for the control pots. One lot of inoculum had been incubated for 14 days and another for 28 days. The data from this experiment are presented in Table I, and also statistical analysis of the data, according to the Analysis of Variance method of Fisher (1), in Table IV.

The seedlings in both sterilized and unsterilized soil series were severely diseased, but the damage was greater in the former. It was found that in both sterilized and unsterilized soil series, the amount of corn meal in the inoculum, the size of flask used to incubate it, and the period of incubation caused significant differences in the number of surviving plants, weighted infection ratings, and weighted dry weights. The inoculum containing 12% corn meal caused more disease than that containing 5%. Also, the inoculum incubated in litre flasks, in 600 gm. quantities, caused slightly more disease than that incubated in 200 ml. flasks. Moreover, the inoculum incubated for 14 days was considerably more pathogenic than that incubated for 28 days. However, with regard to the observed infection rating and the actual dry

weight data, significant differences appeared only in the case of inocula of different ages.

TABLE I

EFFECT OF AMOUNT OF CORN MEAL, SIZE OF CONTAINER, AND PERIOD OF INCUBATION ON THE VIRULENCE OF ARTIFICIAL INOCULUM OF *Helminthosporium sativum* ON WHEAT SEEDLINGS. GREENHOUSE, JANUARY AND FEBRUARY, 1940.

Corn meal, %	Flask, ml.	Incubation, days	Number surviving plants, av.	Average infection, %		Average dry weight, mg.	
				Observed	Weighted	Observed	Weighted
(A) Sterilized soil							
5	1000	14	4.5	60.6	92.7	23.3	5.7
5	1000	28	11.5	70.2	81.7	29.1	17.5
5	200	14	10.2	75.7	86.5	29.3	16
5	200	28	13	63.1	74	32.9	23
12	1000	14	2.7	80.9	97	20	3
12	1000	28	5.2	76.2	93.2	21.8	6
12	200	14	6.2	78.8	92.7	22	7.2
12	200	28	9	56.7	83	34.7	16.7
5	Control	Control	18.7	3.9	3.9	71.3	71.3
12	Control	Control	18.5	3.8	3.8	67.1	67.1
(B) Unsterilized soil							
5	1000	14	4.5	61.1	91	45.6	10.5
5	1000	28	9.7	48.7	69.7	45.8	23.5
5	200	14	9.2	53.1	77	36.8	18
5	200	28	12.7	31.2	52.5	46.9	31.5
12	1000	14	4.7	71	92.7	25.8	6.2
12	1000	28	5.7	56.5	87	38.7	13.5
12	200	14	6.5	78.8	91.7	27.7	9.7
12	200	28	5.5	47.3	84.7	44.1	12.7
5	Control	Control	19.2	5.8	5.8	60.3	60.3
12	Control	Control	18.7	5.9	5.9	53.3	53.3

Experiment II

Plantings were made in April and both *H. sativum* and *F. culmorum* were used as pathogens. In order to prevent harmful loss of moisture from the media, waxed paper caps were fitted on all flasks after sterilization. The inoculum was incubated for 14 and 35 days. Thirty grams of it was applied per pot in the steam sterilized and unsterilized soil series. The data are shown in Tables II and IV.

Severe infection resulted from both *H. sativum* and *F. culmorum*. There were no significant differences in disease severity between the various treatments in the steam sterilized and unsterilized soil in the *H. sativum* series. However, it is possible that the general severity of the disease may have masked differences which would have been noticeable with less disease

In the *F. culmorum* series, with few exceptions, the amount of corn meal in the inoculum, and the period of incubation caused significant differences in number of surviving plants, observed and weighted infection, and the observed and weighted dry weights. Inoculum containing 12% corn meal

TABLE II

EFFECT OF CONTENT OF CORN MEAL, SIZE OF CONTAINER, AND PERIOD OF INCUBATION ON THE VIRULENCE OF ARTIFICIAL INOCULUM OF *Helminthosporium sativum* AND *Fusarium culmorum* ON WHEAT SEEDLINGS. GREENHOUSE, APRIL AND MAY, 1940.

Corn meal, %	Flask, ml.	Incubation, days	Number surviving plants, av.	Average infection, %		Average dry weight, mg.	
				Observed	Weighted	Observed	Weighted
(A) H. sativum, sterilized soil							
5	1000	14	9	55.5	77.7	43.5	20.5
5	1000	35	6.5	50	85.2	44.7	15.5
5	200	14	9.2	60.6	78.2	52.2	26
5	200	35	10.5	50.9	71.5	44.5	26.2
12	1000	14	10.5	61.7	81	34.5	19.5
12	1000	35	5.7	48.7	84.2	49.5	10.7
12	200	14	3.7	81.3	96	26.7	5.2
12	200	35	9.2	35.7	67.7	58	28.7
5	Control	Control	18	5.3	5.3	64.2	64.2
12	Control	Control	18.7	14.4	14.4	75	75
(B) H. sativum, unsterilized soil							
5	1000	14	12.7	36.1	49.2	86	68.2
5	1000	35	6.5	32.3	72.7	100	39.7
5	200	14	7	38.8	73	80.2	35.7
5	200	35	6	30.4	73.7	64.7	33
12	1000	14	10.2	28.3	53.7	70.2	46
12	1000	35	8	33.3	66.7	71.5	36.5
12	200	14	5.7	50	81.7	79.5	29
12	200	35	11.2	20	43.2	86.2	59.2
5	Control	Control	16	36.7	36.7	80	80
12	Control	Control	16	25.8	25.8	59.2	59.2
(C) F. culmorum, sterilized soil							
5	1000	14	5.7	57	85	28	8.5
5	1000	35	6	42.9	89.5	28.2	8.7
5	200	14	7.5	46.7	77.7	30.5	13.7
5	200	35	5.2	56.3	87	19.2	5.2
12	1000	14	4.7	73.7	86	16.2	4.7
12	1000	35	2.7	49.1	94.2	18.7	3
12	200	14	3.5	54.3	91.2	42	7.5
12	200	35	2.2	84.4	97.7	20	2.2
5	Control	Control	18	5.3	5.3	64.2	64.2
12	Control	Control	18.7	14.4	14.4	75	75
(D) F. culmorum, unsterilized soil							
5	1000	14	8.7	27.4	60.2	50.2	27.7
5	1000	35	4.2	39.4	81.2	31.7	9
5	200	14	8	18.4	59.2	44.5	21.5
5	200	35	6.2	35.3	74.7	43	15.7
12	1000	14	2.7	20	86	41.2	6.7
12	1000	35	3.2	68.5	93.5	20	3.7
12	200	14	5.7	18.7	73.3	34	15
12	200	35	3.5	45	87.7	28.2	6.5
5	Control	Control	16	36.7	36.7	80	80
12	Control	Control	16	25.8	25.8	59.2	59.2

was more pathogenic than that containing 5%. The *F. culmorum* inoculum of five-weeks' incubation caused more disease than that incubated for two weeks. The size of container in which the inoculum was incubated had no significant effect on virulence in this experiment.

Experiment III

This test was undertaken to determine whether capping of the flasks during incubation prevented the observed differences in virulence of inocula increased in 1000 and 200 ml. flasks, and to determine also whether possible differences

TABLE III

EFFECT OF CONTENT OF CORN MEAL, SIZE OF CONTAINER, AND PERIOD OF INCUBATION ON THE VIRULENCE OF ARTIFICIAL INOCULUM OF *Helminthosporium sativum* AND *Fusarium culmorum* ON WHEAT SEEDLINGS. GREENHOUSE, JUNE AND JULY, 1940.

Corn meal, %	Flask, ml.	Incubation, days	Number surviving plants, av.	Average infection, %		Average dry weight, mg.	
				Observed	Weighted	Observed	Weighted
(A) H. sativum							
5	1000	14	8.2	48.7	75	118	75.5
5	200	14	9.7	45.5	67	114.2	68.7
12	1000	14	7	52.7	79	73.2	29.2
12	200	14	9.2	43.7	67.5	97.5	57.2
5	1000	21	13	26	41.5	114.7	90.2
5	200	21	15.2	40	45	107	100
12	1000	21	11.2	49.2	64.2	78.7	56
12	200	21	9	44.2	67.7	122.7	68.7
5	1000	28	15	44	49.5	91	82.2
5	200	28	15.5	47.2	50.7	81.2	76.5
12	1000	28	5.7	44.2	79.2	49.2	17.7
12	200	28	4.7	38.7	82.7	85	18.2
5	1000	35	15	33	38.7	68.5	61
5	200	35	18.2	27.5	27.5	103.5	103.5
12	1000	35	13	34.2	47.2	77.7	62.2
12	200	35	5.5	26.7	74.2	89	35
(B) F. culmorum							
5	1000	14	12.7	22.2	39.5	71.5	53.5
5	200	14	12.7	28	44.5	62	46.2
12	1000	14	11.5	39.7	55.5	53	37.7
12	200	14	9.2	19.2	50	85.5	50.7
5	1000	21	13.2	17	33.5	88.7	70.7
5	200	21	14.7	18.7	28.5	67	60.5
12	1000	21	12.5	23.2	37.7	57.2	45.7
12	200	21	9.7	28.2	54	81.5	51.7
5	1000	28	14.5	22.7	32.7	84.5	70.5
5	200	28	13.7	15.2	28	80.2	67.5
12	1000	28	15.2	18	24	98	96.5
12	200	28	11.7	24.7	43.7	75.2	61.2
5	1000	35	15.2	25.5	31.7	63.2	57.2
5	200	35	15.5	22.2	26.7	54.7	51.5
12	1000	35	11	30.7	51	40.5	28.5
12	200	35	11.7	32.5	49.7	63.2	46
5	Control	Control	16.5	8	8	102	102
12	Control	Control	15.5	9.5	9.5	88	88
12	Control	Control	17.4	8.4	8.4	92.6	92.6

TABLE IV

F. VALUES* OF DATA FROM TABLES I, II, AND III

Variable	Number surviving plants	Average infection		Average dry weight	
		Observed	Weighted	Observed	Weighted
Table I, A					
Amount corn meal	22.53	3.97	13.28	.05	16.88
Size container	18.53	1.53	11.22	1.78	18.65
Days incubated	19.80	5.79	18.92	17.65	19.26
Table I, B					
Amount corn meal	22.94	30.28	25.91	3.12	30.11
Size container	7.88	1.64	7.08	.05	5.67
Days incubated	8.84	35.45	20.35	4.75	15.48
Table II, A					
Amount corn meal	1.79	.00	1.36	.55	3.22
Size container	.00	.00	1.12	.18	2.23
Days incubated	.00	9.69	3.03	3.31	.54
Table II, B					
Amount corn meal	.40	.08	.86	3.30	.04
Size container	2.50	.08	1.35	.44	.12
Days incubated	.71	6.95	.00	1.78	1.19
Table II, C					
Amount corn meal	24.63	5.58	10.81	.27	10.07
Size container	.01	.79	.00	1.39	.45
Days incubated	4.98	1.00	9.76	3.08	6.70
Table II, D					
Amount corn meal	16.43	2.80	21.42	4.87	16.65
Size container	2.31	2.53	3.25	.09	1.24
Days incubated	7.30	24.70	16.80	5.19	12.23
Table III, A					
Amount corn meal	36.85	1.27	52.80	4.88	54.35
Size container	.00	.89	.13	5.20	1.61
Days incubated	4.56	9.21	15.20	3.80	5.74
Table III, B					
Amount corn meal	21.89	6.42	17.95	.12	2.28
Size container	2.48	.34	.67	.07	.40
Days incubated	3.54	3.02	4.46	39.30	6.96

* *Tables I and II. 5% point, 4.17; 1% point, 7.56.*
Table III. Amount corn meal: 5% point, 4.00; 1% point, 7.08.
Size container: 5% point, 4.00; 1% point, 7.08.
Days incubated: 5% point, 2.76; 1% point, 4.13.

from the various treatments might become evident under less severe infection than occurred in the *H. sativum* series of Experiment II.

The flasks were again capped with waxed paper in order to eliminate the desiccation factor. Also, to diminish the degree of infection, only 20 gm. of inoculum per pot was used. The inoculum was incubated for periods of 14, 21, 28, and 35 days to give a greater range of the age factor. In this experiment, only unsterilized soil was used in the pots. Two kinds of controls were employed, namely, pots with unseeded medium plus unsterilized soil, and

those with unsterilized soil only. These two types of controls were expected to indicate the adverse effect of added carbohydrate material. The results appear in Tables III and IV.

In the *H. sativum* series, significant differences for effects of varying amounts of corn meal and of various periods of incubation were found in almost all cases. The inoculum containing 12% corn meal was, as in previous experiments, more pathogenic than that containing 5%. The 14-day-old inoculum caused more disease than that 35 days old, whereas the results from the inoculum incubated for 21 and 28 days were intermediate.

In the *F. culmorum* series, significant differences were found in almost all cases for the effects of the amount of corn meal added and the period of incubation. The inoculum containing 12% corn meal was more pathogenic than that with 5%. The inocula incubated for 14 and 35 days were about equally pathogenic and somewhat exceeded those in the 21- and 28-day series in causing disease. The size of container did not affect the virulence of the inoculum.

Discussion

The results presented emphasize the importance of standardized technique in the preparation of media in which to increase certain soil-inhabiting pathogens. The amount of organic matter incorporated in the medium affects the virulence of inoculum of *H. sativum* and *F. culmorum*. If soil is used as a carrier, the data presented here indicate that in order to prevent organic matter from causing increased virulence, the amount of carbohydrate added should be a minimum commensurate with good growth of the pathogen.

Also, under certain conditions, differences of only one week in the period of incubation of the inocula of *H. sativum* and *F. culmorum* may noticeably affect their virulence; greater differences in age would be expected to produce more marked effects, as Sallans (2) has shown for oat hull medium.

Further, the virulence of the inoculum may be affected by the amount of medium seeded to the pathogen, if the physical conditions of storage are such that irregularities in the rate of water loss, and in air humidity within the flasks, occur as a result of larger differences in the volume of container or medium. Also, excessive desiccation of inoculum can occur, especially in small flasks, under conditions existing in most laboratories; control of this factor is advisable.

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STUDIES ON BACTERIOPHAGE IN RELATION TO CHEDDAR CHEESEMAKING[1]

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Abstract

The sudden stoppage of acid development in several experimental vats of Cheddar cheese was shown to be due to the activity of a polyvalent streptococcal phage. Although the starter used consisted of a mixture of organisms, the stoppage was as abrupt as that in cases in which single strain starters are employed. Experimental infection of a vat of milk duplicated the original findings and resulted in the production of a cheese of poor body and texture, with a decidedly "fruity" flavour.

The presence of phage in the starter itself could not be demonstrated. Tests made on the milk of 18 cows, composite herd samples, stable and laboratory air, and rennet extract failed to indicate specifically the origin of the lytic agent.

Introduction

In the manufacture of Cheddar cheese a uniform development of lactic acid throughout the process is highly desirable. To ensure this, a quantity of "starter" consisting of a clotted milk culture of lactic acid streptococci is added to the milk. Failure of the starter to develop acid at the required rate disrupts the normal manufacturing process, causes considerable inconvenience, and lowers the quality of the cheese.

In New Zealand, failure to produce acid has been sufficiently common to justify extensive research directed to the elimination of this trouble. Whitehead and Cox (11, 12) have shown that such failures were frequently due to the sudden development of streptococcal bacteriophages that lysed the starter organisms and prevented the normal fermentation of lactose. Since the starters used in New Zealand are mainly prepared from single strains of *Streptococcus cremoris* (13), the appearance of a phage specific for the particular strain of streptococcus present in the starter results in complete lysis of this organism, either in the starter itself or at some stage in the manufacturing process. In view of the strain-specific nature of these streptococcal phages, the presence of phage rarely, if ever, results in as complete a failure of acid production with a mixed culture as it does with a single-strain starter (15).

Streptococcal phages are a common phenomenon in New Zealand (13), and they have also been demonstrated elsewhere. Mazé (5) has reported the presence of phages attacking the "normal lactic acid bacteria" in cheese factories in France. Davis (1) has recently reported the first case of phage activity in England, where single-strain cultures were being used. Nelson *et al.* (7) in Iowa have shown that slow acid production in butter cultures may be due to phage activity, but we have failed to find any reference to phage in

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connection with cheesemaking in North America. However, in the opinion of an outstanding authority (8), troubles attributable to phage may be much more general than is suspected. In view of the possible economic importance of the problem, the following account of an outbreak of phage infection at the Central Experimental Farm, Ottawa, may be of interest.

Description of the Outbreak

Acid development ceased abruptly in the early stages of manufacture in three vats of experimental cheese; in the fourth vat it slowed up later, the "milling" process being three hours behind the normal. Microscopic examination of the whey from these vats, after the stoppage occurred, revealed the complete disappearance of the starter oganisms.

The starter employed, No. 21, consists of a mixture of various types of streptococci that may be distinguished from one another by length of chain formation, rate of acid production, and other physiological characteristics (Table I). It had been collected from a factory in Eastern Ontario in October, 1939 (2). Because of its strong acid production at 37° C., this starter was selected for distribution to cheesemakers throughout Eastern Ontario. During the five months up to the end of October, 1940, more than 250 cultures prepared from this starter had been supplied to 162 factories. This starter has also been employed continuously at the Central Experimental Farm Dairy for the ripening of cream for buttermaking, and in the manufacture of more than 60 vats of experimental cheese. At no other time had any weakening in its fermentative power been observed, nor has there been any word of this from the numerous factories using it.

Evidence of Phage Activity

That the abrupt cessation of acid production in the experimental vats was due to phage activity was first indicated by the disappearance of the starter organisms from the whey. Further confirmation was obtained in several ways. (1) Whey was passed through a sterile Seitz filter; a drop of diluted filtrate, when added to sterile milk freshly seeded with starter No. 21, caused virtually complete disappearance of the starter organisms within four and one-half hours' incubation at 32° C., whereas the control tube showed millions of streptococci per millilitre (Figs. 1, 2, and 3). The lytic agent could be transmitted indefinitely by inoculation into fresh milk seeded with this starter. (2) Plaque formation on yeast-extract–whey agar (Fig. 4) was demonstrated by means of the technique described by Whitehead and Cox (12). (3) Lysis in broth was demonstrated by the addition of aliquots of the whey filtrate to yeast-extract–whey broth cultures of 10 organisms isolated from this starter. As will be seen from Table I, 7 of the 10 cultures were completely lysed within six hours, while the remaining three showed partial lysis. (4) The substitution of a new starter (No. 2) for No. 21 in the experimental cheesemaking immediately following the phage attack resulted

TABLE I

SENSITIVITY TO LYSIS, MORPHOLOGY, AND PHYSIOLOGICAL CHARACTERISTICS OF STRAINS OF STREPTOCOCCI ISOLATED FROM STARTER No. 21

Strain No.	Degree of lysis*						Morphology**	Physiological characteristics						
	Incubation, hr.							Growth in			Growth at			NH_3 produced from peptone
								Litmus milk		4% NaCl broth	pH 9.1	10° C.	40° C.	
	2	3	4	5	6	7		Reduction	Coagulation					
1	−	+++	++++	++++	++++	++++	Long and short chains	+	+	+	+	+	+	+
6	−	−	++++	++++	++++	++++	Very long chains	−	−	+	+	−	−	+
7	−	−	−	+	+	++	Pairs and short chains	Slight	−	+	+	+	−	+
8	−	+++	++++	++++	++++	++++	Pairs and short chains, some long chains	+	+	+	+	+	+	−
10	−	+++	++++	++++	++++	++++	Pairs, no chains	+	+	+	−	+	+	+
12	−	−	+	+	+	+	Long chains, a few short chains	+	+	+	−	+	+	−
15	−	+++	++++	++++	++++	++++	Long chains	+	+	+	−	+	+	+
16	−	−	+	+	+	+	Long and short chains	+	+	+	−	+	+	+
21–1	−	−	+++	+++	++++	++++	Short to fairly long chains	+	+	+	−	−	+	+
21–2	−	−	++	+++	++++	++++	Pairs, a few short chains	+	+	+	+	+	+	+
Controls	−	−	−	−	−	−								
S. lactis							Pairs and short chains	+	+	+	+	+	+	+
S. cremoris							Long chains	+	+	−	−	+	−	−

** *Milk cultures after 16 hr. at 22° C.*

* *Degree of lysis:* ++++ = *complete.*
+++ = *almost complete.*
++ = *decidedly clearer than control.*
\+ = *slightly clearer than control.*
− = *same as control.*

in perfectly normal acid development in the next 10 vats. (5) The ability of an active phage filtrate to stop acid production in the cheese vat was demonstrated experimentally. A small vat containing 400 lb. of milk was inoculated with 4 lb. (1%) of a clotted milk culture of starter No. 21. After thorough mixing, one-half of the milk was transferred to an identical vat. Both vats were handled as uniformly as possible except that the wooden rake used to agitate the milk and curd in one vat, *F*, was lightly swabbed with a little of the phage-containing filtrate. The control vat (*C*) behaved normally throughout and produced a first grade cheese. On the other hand, in the phage-contaminated vat, acid development ceased immediately after cutting. The first titration of the whey showed 0.13% acidity; four hours later the acidity was unchanged. Microscopic preparations and Burri slants prepared from both vats shortly after the stoppage was noted showed millions of starter organisms in the whey of the control vat, none at all in that of vat *F*. Although some slight acid development, due to the action of non-starter organisms, was noted many hours later in vat *F*, the cheese was practically ruined.

After holding for 18 days at 15° C., the two vats were scored as follows*:

Vat	Flavour	Texture	Closeness	Colour	Finish	Total score
Control *(C)*	39.5	24.2	15.0	10.0	5.0	93.7
Filtrate *(F)*	37.0	23.0	14.5	9.5	5.0	89.0

* *The Dominion Grade Standards specify the following minimal scores for cheese:*

	Flavour	*Total score*
First Grade	*39*	*92*
Second Grade	*37*	*87*
Third Grade	*<37*	*<87*

The pronounced "fruity" flavour that lowered the score of cheese from vat *F*, appeared to be due to the large number of yeasts in the cheese (1,600,000 yeasts per gm.), whereas that from the control vat (*C*) made from identical milk contained 11,000 per gm. Plates poured on tryptone–glucose–skim-milk agar containing brom-cresol purple indicator showed a typical lactic acid flora in the control cheese (vat *C*) but almost complete absence of these organisms in the phage contaminated cheese (Figs. 5 and 6).

Further Studies with Phage

It has been previously mentioned that the bacteria-free filtrate of the original whey was active against all 10 strains of organisms isolated from starter No. 21. To determine whether the cause of the trouble was an unusually polyvalent phage or a mixture of races, a lysed yeast-extract–whey broth culture of Strain 8 was filtered through a sterilized Seitz filter, and the filtrate added to a freshly seeded broth culture of the same organism. This process was

TABLE II

EFFECT OF ADDING ACTIVE FILTRATE FROM STRAIN 8 TO 11 STOCK STARTERS, AS SHOWN BY MICROSCOPIC EXAMINATION OF MILK CULTURES AFTER SIX HOURS AT 32° C.

Starter No.	Control	Filtrate added	Morphology
1	++++*	++++	Pairs and short chains
8	++++	++++	Long chains
20	++++	++++	Pairs and short chains
21	++++	—	Long chains
30	++++	++	Pairs and short chains
31	++++	++++	Pairs and short chains
32	++++	++++	Pairs and long chains
33	++++	++	Pairs and short chains
34	++++	++++	Pairs and short chains
35	++++	++++	Pairs and long chains
36	++++	+++	Pairs and short chains

* *Plus signs indicate approximate abundance of organisms observed.*

repeated three times and the filtrate added to sterile milk seeded with starter No. 21. After eight hours' incubation, the organisms had completely disappeared. Similar results were obtained after 12 additional serial passages of the active filtrate at the expense of Strain 8. This would indicate that the active agent was a polyvalent phage rather than a mixture of races, as it would be expected that 15 serial transfers would have diluted out any races not specific for Strain 8.

To obtain further proof of the polyvalency of this lytic agent, the filtrate from the 15th transfer was tested against broth cultures of the original 10 strains (Table I). Seven of these (No. 6, 7, 8, 10, 15, 16 and 21-1) showed evidence of lysis. These represent organisms of varying morphology and physiological reactions (Table I). This reinforces the evidence mentioned in the previous paragraph suggesting that the phage in question is polyvalent.

To discover whether the phage would affect streptococci in other mixed culture starters the sterile filtrate from a lysed culture of Strain 8 was tested against milk cultures of 11 starters being carried in these laboratories. The results are presented in Table II. It will be observed that in three of these cultures there was evidence of inhibition of growth. Although a milk culture of starter No. 21 is characterized by the presence of very long chains of streptococci, the activity of this phage was not confined to strains with this characteristic. Starters No. 30, 33, and 36 contained predominantly short-chain streptococci, yet all three showed reduction in numbers of organisms, whereas No. 8 and 35, predominantly long-chain types, showed no such decrease. This is in accord with the results reported in Table I, where strains of both long and short-chain streptococci were completely lysed.

The potency of the phage present in a lysed culture of Strain 8 was determined, using decimal dilutions in yeast-extract–whey broth seeded with a broth culture of the susceptible organism. The following results were obtained:

Incubation period, hr.	Concentration of filtrate							
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
	Degree of lysis							
6	++++	++++	++++	++++	+++	+++	+	−
18	++++	++++	++++	++++	+++	++	+	−
24	++++	++++	++++	++++	+++	++	++	−

In the cheese factory it is customary to use the same container for measuring out both cheese colour and rennet. After adding the colour to the milk just before "setting" the vat with rennet, the container is customarily rinsed in the milk, then in water. If phage is present in the milk, the container is quite likely to become contaminated. Rennet extract is next poured into the container, and, if an excess is introduced, the surplus is poured back into the vessel holding the rennet supply. In this way the rennet itself may become contaminated with phage. Unfortunately, the rennet extract was not tested for the presence of phage until 10 days after the outbreak; negative findings at that time do not preclude the possibility that the phage had died out in the interim.

The survival of phage in the cheese itself was investigated. Decimal dilutions of the cheese from vat *F*, after 18 days' ripening at 15° C., were added to sterile milk tubes seeded with starter No. 21; subcultures were later made from these. No evidence of lysis was obtained even in the lowest dilution (10^{-1}); this suggested that the phage had died out during this period.

Source of the Phage

The actual source of these streptococcal phages is still a matter of opinion. Whitehead and his co-workers (12, 13, 14) concluded that the streptococcal phages originate within the single strain starters themselves. External contamination was ruled out mainly on the grounds of the relatively high degree of specificity exhibited by the races of phage they studied. In a more recent publication (16), however, reference is made to the possible contamination of starter cultures with phage within the factory, since cheese whey commonly contains a high concentration of phage (14). Mazé, on the other hand, while confirming the specificity of the races of phage, believes the trouble arises from external contamination within the cheese factory (6). While admitting that the origin of the phage is still obscure, he suspects the raw milk because of its frequent contamination with organisms from manure (5). Since starter organisms (*Streptococcus lactis* and *S. cremoris*) are not found in bovine faeces (9), it seems unlikely that this would be an important source of the phage specific for these organisms.

Starter

In view of the emphasis placed by the New Zealand workers upon the starter itself as the source of phage, a number of attempts were made to

demonstrate its presence. There is evidence that some milks favour the development of phage whereas others appear to inhibit it (12, 13). It has also been shown that conditions (use of small inocula, aeration of milk) that prolong the lag phase of growth of the starter organisms, facilitate the demonstration of phage that may be present in the starter. Consequently, 125 ml. portions of milk were obtained from each of 12 cows in various stages of lactation, and with udders of varying degrees of abnormality. These milks were autoclaved at 15 lb. pressure for 20 min.; after cooling to 30° C., they were lightly seeded with a six-day-old starter culture and aerated aseptically by passing a stream of air through the flasks for 15 hr. Microscopic preparations showed abundant growth in every case. This was also true for tubes of sterile milk inoculated from the 15 hr. flask cultures. These all clotted normally, as did a second set of milk tubes inoculated from the first.

Tests were also made with a number of autoclaved or pasteurized samples representative of the milk of the entire herd. In only one instance was there any indication of the presence of phage. In the one exception, 400 ml. of milk pasteurized at 62° C. for 30 min. was seeded with a five-day-old starter culture. After incubation for one hour, calcium chloride solution and rennet extract were added to coagulate the milk. The whey that extruded on standing at 37° for 30 min. was filtered through paper, then through a sterilized Seitz filter. Three-millilitre portions of whey filtrate were added to yeast-extract–whey broth cultures of the 10 organisms reported in Table I. After six hours' incubation, Strains 8 and 15 showed lysis.

This finding cannot, however, be regarded as proof of the presence of phage in the starter itself. In the first place, the heat treatment employed in pasteurization would not destroy phage present in the raw milk (13). Secondly, the rennet had been exposed to phage contamination in the manner described in a previous section. Thirdly, there exists the possibility of accidental contamination. A repeat test was made a few days later using milk steamed for 45 min. and aerated aseptically during incubation for six hours with the starter inoculum. No lysis was noted when the whey filtrate was tested against a broth culture of Strain 8, nor when this culture was filtered and added to a fresh culture. As previously mentioned, phage could not be demonstrated in the rennet extract.

Negative results were also obtained with several samples of milk from the factory supplying the milk in which the phage activity was first demonstrated. The vitality test of Whitehead and Cox (10) was conducted concurrently on portions of milk from both normal and abnormal vats on the day that trouble was experienced. Although the same lot of starter was employed as in the vats in which phage developed, there was no evidence of phage action in the vitality test.

That the milk supply ordinarily used in preparing the starter was suitable for the demonstration of phage active against starter No. 21 has been indicated earlier. Repetition of the first experiment by adding low dilutions of active

filtrate to sterile milk inoculated with starter cultures from three to six days old gave identical results, i.e., complete disappearance of starter organisms.

Milk Supply

While it seems unlikely that milk from individual cows would contain phage active against starter No. 21, tests were conducted upon samples obtained from 18 cows in the Central Experimental Farm herd. Samples obtained from the milkers' pails were seeded lightly with an old culture of starter and aerated aseptically overnight. Next morning, transfers were made from the original samples to sterile milk tubes freshly seeded with the starter; a second transfer into similar milk tubes was made from the first tubes after eight hours' incubation at 32° C. Although none of the original samples showed any evidence of phage activity, the tube transfers from two of these milks did show lysis. Seitz filtrates obtained from these were added to sterile milk seeded with starter and lysis was again noted. To confirm the presence of phage in the milk of these two cows, further samplings of aseptically drawn foremilk and of milk from the pail were tested at 20 subsequent milkings. In no instance was there any evidence of phage.

Air

Tests for the presence of phage in the stable air were made upon two occasions by exposing a 15 sq. in. surface of sterile milk for 10 to 20 min. during milking. The results were negative. A similar exposure test in the laboratory (four weeks after the outbreak) yielded negative results.

Discussion

The outbreak of phage infection described is of particular interest in that the stoppage of acid fermentation in vats seeded with a mixed starter was as abrupt and complete as any reported for single strain starters. As Whitehead and Hunter (15) have remarked, this rarely, if ever, happens. In New Zealand, cheese milk is flash-pasteurized to destroy the bulk of contaminating bacteria before the starter is added. If the phage attacking the single strain culture of *Streptococcus cremoris* is present in amounts sufficient to lyse these organisms completely, subsequent acid production would necessarily be very slow. Although the milk in our experimental vats was not pasteurized, the bacterial content was fairly low, only one of the four vats reducing resazurin to the pink stage (3) within five hours. Consequently, when the various strains of streptococci in the starter were lysed, there was no further acid development until the contaminating organisms present in the original milk (mostly micrococci) reached sufficient numbers.

In view of the New Zealand findings, it is also noteworthy that we have failed to demonstrate with certainty the presence of phage in the starter itself, when grown in a variety of milk supplies, and under conditions conducive to its appearance. However, in view of Whitehead and Hunter's (13) observations concerning the "periodic sensitivity" of starters for phage demonstration, it

may not be safe to conclude that the starter in question is at all times completely free from phage.

The writers' inability to demonstrate phage in so many subsequent samplings of milk from the two cows in whose milk phage was detected on a single occasion leads to the suspicion that the positive findings were due to accidental contamination during the inoculation and subsequent transfers of these two samples. The ease with which phage contamination may occur in a laboratory is well known (4). In a recent report (16) Whitehead and Hunter remark, "A few further experiments served to show quite clearly that, in the commercial factories, contamination of the cultures with phage occurred through the atmosphere even when a normal bacteriological technique was practised . . . . . It is evident now that the technique normally considered adequate to prevent infection of cultures is not efficient under certain circumstances in preventing the access of bacteriophage to cultures."

The original source of the phage in the present outbreak thus remains obscure. Milks from a variety of sources had been employed in experimental cheesemaking just prior to the outbreak. Any one of these supplies may have introduced the phage, which would then have been carried over from day to day on the wooden rakes and other equipment. Since it could not be ascertained which herds furnished these supplies further investigations in this direction could not be pursued.

Slow acid development is not uncommon in Eastern Canada although no previous investigations regarding phage activity have been reported from this area. Cheesemakers admit having experienced trouble which they have attributed to some inexplicable failure of the starter. The substitution of a different starter generally corrected the trouble and the cheesemaker's diagnosis appeared to have been fully substantiated. That the fault may not be in the starter itself, however, is suggested by the present experience. If the maker experiencing trouble due to phage activity happens to replace his starter with a culture of similar bacterial composition, he will almost inevitably experience further trouble and unjustly blame the new starter. This might easily happen with a maker using No. 21, which is still being distributed regularly. That the trouble may not be inherent in the starter itself should be impressed upon all cheesemakers, lest the possible consequences prove embarrassing to those responsible for the distribution of starters.

While such an abrupt and complete stoppage of acid development as encountered by the writers may not be common in commercial factories, slow acid development, frequently resulting in "fruity" flavour, is encountered occasionally. The writers' experiences, in which three out of five phage-contaminated vats were graded down for "fruity" flavour, suggest that phage contamination may be concerned in the slow acid development. Where this occurs, it would be wise to change to a different starter, and to subject the premises and equipment to a very thorough cleaning, followed by sterilizing treatment with a hypochlorite solution. This procedure has been reported as effective in

eliminating the trouble in several instances (8). Further studies dealing with this phase of the subject are projected.

That air-borne contamination with phage is a significant factor is indicated in a report from New Zealand just published (17). By the provision of a special starter-room separate from the factory, failures of starters due to phage contamination were completely eliminated at this particular factory throughout the season. The centrifugal separator used in removing the butterfat from the whey was found to emit finely atomized whey and this proved to be the major source of the air-borne phage in the factory.

Acknowledgments

The writers are greatly indebted to Mr. C. A. Gibson, Creamery Manager, Central Experimental Farm, and to Dr. E. G. Hood of this Division, for help in the practical demonstration of phage activity in the cheese vat; to Dr. H. L. Bérard of this Division for making available cultures and information concerning starter No. 21; and to Mr. T. J. Hicks, Senior Dairy Produce Grader, Dairy Products Grading and Inspection Services, Marketing Service, Department of Agriculture, for scoring the cheese.

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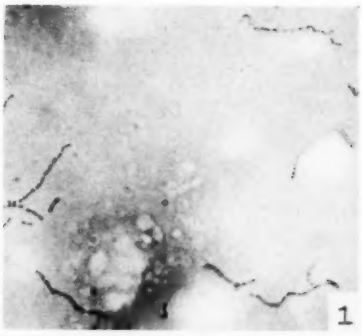
EXPLANATION OF PLATE I

FIG. 1. *Sterile milk inoculated with starter No. 21; before incubation.* FIG. 2. *Same milk after four and one-half hours' incubation at 32° C.* FIG. 3. *Similarly inoculated milk plus phage after four and one-half hours' incubation at 32° C.*

(FIGS. 1 – 3. *Photomicrographs of microscopic preparations, Breed-Newman stain.* × *1000.*)

FIG. 4. *Portion of plate culture of starter No. 21 seeded with active filtrate, showing phage plaques. Natural size.* FIG. 5. *Plate poured from* 1×10^{-7} *dilution of cheese from control vat. Tryptone–glucose–skim-milk agar; incubation period, five days at 30° C. Natural size.* FIG. 6. *Plate poured from* 1×10^{-7} *dilution of cheese from phage contaminated vat. Tryptone–glucose–skim-milk agar; incubation period five days at 30° C. Natural size.*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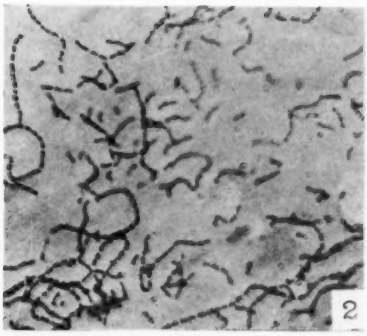
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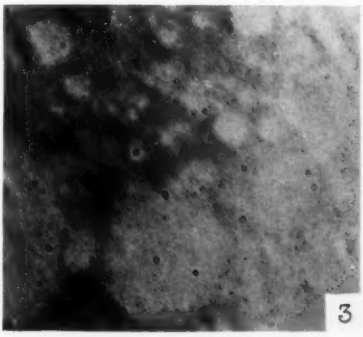
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EFFECT OF TEMPERATURE AND HUMIDITY ON COLOUR OF LEAN, AND DEVELOPMENT OF RANCIDITY IN THE FAT, OF PORK DURING FROZEN STORAGE[1]

BY W. H. COOK[2] AND W. HAROLD WHITE[2]

Abstract

Pork cuts were stored at relative humidities of 83, 87, 92, 96, and 100% and temperatures of −6.6°, −12.2°, −17.7°, and −23° C. for 48 weeks. Visual examination revealed various degrees of surface drying under all conditions, and pronounced methaemoglobin formation at −6.6° C. Quantitative colour measurements on both the exposed and internal surfaces of the lean, showed that storage temperature was the primary factor affecting the colour. Temperatures of −18° C., or lower, are required to prevent these changes. Samples that had suffered the greatest colour change during storage showed the least change during subsequent exposure.

Temperature was also the primary factor affecting the development of rancidity in the fat. Both peroxide oxygen and free fatty acid increased significantly with increase in storage temperature, particularly between −12.2° and −6.6° C., but the actual quantities of free fatty acid were small and of little consequence. Storage temperatures of −18° C., or lower, are essential if spoilage of pork fat is to be avoided over storage periods of approximately one year's duration.

Introduction

In Canada considerable quantities of pork must be stored in the frozen condition for the subsequent manufacture of Wiltshire bacon during periods when hog receipts are limited. Since this material must be thawed, cured, and exported after storage, rather exacting storage conditions are required. Microbial activity, methaemoglobin formation, and the development of rancidity in the fats are the principal forms of deterioration to be expected at the higher freezer temperatures. The low relative humidity prevailing in most freezers also causes surface drying, the condition responsible for freezer burn and loss of bloom. This investigation was undertaken to obtain quantitative information on the change of bloom and pigment in the lean, and spoilage of the fat, of pork stored under various temperature and humidity conditions.

[1] *Manuscript received November 5, 1940.*

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. A portion of the material was presented by one of the writers (W.H.W.) in a thesis to the University of Western Ontario in partial fulfilment for the degree of Master of Arts. Issued as paper No. 57 of the Canadian Committee on Storage and Transport of Food and as N.R.C. No. 966.

[2] *Biochemist, Food Storage and Transport Investigations.*

Methods

The method employed for maintaining the required temperature and humidity conditions was that used in similar investigations on poultry (1). Small cuts of prime pork back were placed in desiccators containing calcium chloride solutions of such concentrations as to maintain the desired relative humidities. The containers were stored in refrigerated rooms at the desired temperatures. Every precaution was taken to insure that the effective relative humidity in each container corresponded as closely as possible to that computed from the vapour pressure of the calcium chloride solution. All surfaces of the pork that could not be seen during inspection were covered with waxed paper to prevent evaporation. Since small samples were used, the area of evaporating surface was negligible compared with the exposed area of the solution; thus it was insured that the humidity corresponded to that of the solution, and excessive dilution of the solution during storage was avoided. To avoid condensation on the inner surfaces, all samples were frozen before they were placed in the containers.

Duplicate samples were stored at 83, 87, 92, 96, and 100% relative humidity at each of the storage temperatures −6.6°, −12.2°, −17.7°, and −23° C. (20°, 10°, 0°, and −10° F.). The values of the relative humidity represent the means of the initial and final values as computed from the corresponding density measurements.

The colour measurements were made with the colour comparator previously described (4). A single measurement was made on each exposed surface and duplicate measurements on internal surfaces. An estimate of colour stability was obtained from measurements on the internal surfaces after exposure for 17 and 89 hr. at 10° C. and 95 to 100% relative humidity.

Peroxide oxygen and free fatty acid determinations were made on samples of the fat prepared by procedures to be described in detail in another paper. In brief, chopped samples of the fat, after drying by admixture with anhydrous sodium sulphate, were extracted in a Soxhlet apparatus for two hours with redistilled petroleum ether. Most of the ether was subsequently removed by distillation, and final traces by heating *in vacuo* at 40° C. The peroxide oxygen and free fatty acid, taken as estimates of oxidative and hydrolytic changes respectively, were determined as previously described (2).

Results of Visible Examinations

Periodic examinations were made during the storage period for surface drying, discoloration, and mould development. A loss of bloom was evident in all samples after 8 to 12 weeks' storage. In the samples stored at low humidities and high temperatures, this condition gradually developed into freezer burn of various degrees of severity. Quantitative estimates of the extent of freezer burn were therefore impossible. Methaemoglobin formation and mould development occurred on all samples stored at −6.6° C. The final examination confirmed these observations and yielded slight evidence of methaemoglobin formation in certain samples stored at −12.2° C.

Colour Measurements

It was felt that colour measurements on the exposed and internal surfaces after storage might permit quantitative expression of the observed changes in bloom and pigment. Although all three colour components, i.e., red, green, and blue, were measured for all samples, it is unnecessary to report the detailed results. It has already been shown that the scatters of the individual components are correlated (6), and although a significant element of independent fluctuation can be demonstrated when a large number of measurements are available, the data obtained in these experiments were considered inadequate for this purpose. This correlation between the components was demonstrated, in these experiments, by the fact that if one component was affected significantly by one of the storage conditions, the other components generally showed the same behaviour. In these circumstances, only the results on the total scatter for all components (brightness) are presented except in a few instances where a single component showed significant effects not revealed by the total scatter. Subsequent tables show the results obtained at each temperature, averaged for all humidities, and those for each relative humidity, averaged for all temperatures. This is supplemented with an analysis of variance for each component separately. A similar treatment was used for all fat measurements.

TABLE I

SURFACE COLOUR OF PORK AFTER STORAGE FOR 48 WEEKS AT VARIOUS TEMPERATURES AND RELATIVE HUMIDITIES

Mean brightness, for all relative humidities at each storage temperature

Temperature, ° C.	− 6.6	−12.2	−17.7	−23.0
Brightness (total, %)	58.5	53.0	46.2	46.6

Mean brightness, for all temperatures at each relative humidity

Relative humidity, %	83	87	92	96	100
Brightness (total, %)	49.1	49.4	51.9	55.0	49.8

Analysis of variance

Variance attributable to	Degrees freedom	Mean square			
		Blue	Green	Red	Brightness (total)
Between temperatures	3	12.9 **	79.5 **	37.7 **	324 **
Between humidities	4	3.11	5.01	8.79	47.8
Residual[1] (T × H)	12	1.79	6.16	6.51	37.8

***Indicates 1% level of significance.*

[1] *Residual (T × H) did not significantly exceed error of duplicates.*

The results reported in Table I show that the samples gradually darkened in colour as the temperature decreased from −6.6° to −17.7° C. There was also some indication that the brightness decreased with relative humidity. The analysis of variance shows that the difference due to storage temperature was the only one that attained statistical significance; all components and their totals showed significant effects.

Table II shows the results of similar measurements on the internal surfaces. Storage temperature was again the primary factor affecting colour, although significant effects could not be demonstrated for the red component. The green component alone showed significant differences following storage at the various humidities, the values being somewhat lower at the two lowest relative humidities. Since the samples were rather small, the effect of surface drying and discoloration may have penetrated in some samples to the depth at which these internal colours were measured. Had it been possible to obtain samples at a considerable depth, significant effects of relative humidity, at least, would hardly be expected.

The colour measurements on the internal samples are of interest for comparing the observed changes in the exposed samples. The measurements on the internal surfaces of the samples stored at −17.7° and −23° C. showed that

TABLE II

INTERNAL COLOUR OF PORK AFTER STORAGE FOR 48 WEEKS AT VARIOUS TEMPERATURES AND RELATIVE HUMIDITIES

Mean brightness and green scatter, for all relative humidities at each storage temperature

Temperature, ° C.	− 6.6	−12.2	−17.7	−23.0
Brightness (total, %)	57.8	51.4	54.1	54.0
Green scatter, %	17.5	15.4	16.2	16.0

Mean brightness and green scatter, for all temperatures at each relative humidity

Relative humidity, %	83	87	92	96	100
Brightness (total, %)	53.1	52.2	55.7	55.8	54.7
Green scatter, %	15.8	15.6	16.5	17.0	16.6

Analysis of variance

Variance attributable to	Degrees freedom	Mean square			
		Blue	Green	Red	Total
Between temperature	3	6.09*	16.5 **	12.6	137 **
Between humidities	4	2.78	7.10*	3.11	40.7
Residual[1] (T × H)	12	1.22	2.20	4.90	38.7

**Indicates 5% level of significance.*

***Indicates 1% level of significance.*

[1] *Residual (T × H) did not significantly exceed error of duplicates.*

the colour was satisfactory, and can be considered as unchanged from the original. The brightness of these samples was about 54%. By comparison, the brightness of exposed surfaces must have increased during storage at −6.6° C., remained constant when stored at −12.2° C., and darkened at the lower storage temperatures. The increase in brightness at −6.6° C. is attributed to methaemoglobin formation, a fact confirmed by observations made on other samples. The darkening observed following storage at −17.7° and −23° C. must be caused by the drying or loss of bloom, the only change observed at these temperatures. Earlier studies (3) have shown that drying at temperatures above the freezing point is accompanied by darkening, and a similar behaviour apparently applies to drying from the frozen state. The value of 53% obtained for the brightness of exposed surfaces of samples stored at −12.2° C. does not differ appreciably from 54% and suggests that the surface of the samples remained unaltered. Since drying did occur on the surface of all samples, darkening would be expected. Apparently sufficient brightening was obtained by methaemoglobin formation to offset the darkening caused by drying. The change in the brightness of the internal samples was less marked, and is doubtless attributable to similar effects.

Colour Stability Measurements

The internal surfaces were exposed, under conditions described previously, for periods of 17 and 89 hr. and again measured. The change from the initial value over each of these periods was taken as an estimate of colour stability. The general tendency was for the samples to become brighter during the 17-hr. period, and to become even darker than the initial value following the 89-hr. period. In expressing and analyzing the results, an increase in brightness is reported as a positive, and darkening as a negative, value.

The initial average brightness, and that following each exposure period, together with an analysis of variance on the change in colour scatter for each component for each period, appears in Table III. Storage temperature was the only factor showing a significant effect on colour stability. There is also evidence of differential effects or changes in colour quality, since the change in green scatter and total brightness after 17-hr. exposure was not related to the previous storage temperature, while the change in the red component was not significant after 89-hr. exposure. For the samples stored at all temperatures, an initial increase in brightness followed by a decrease is evident, but the initial brightness and the extent of the changes vary markedly.

A complete interpretation of these data is impossible at the present time. Presumably the formation of methaemoglobin during storage at the higher temperatures was responsible for the increased brightness. During exposure for a short period at a higher temperature, this process continues, but is least marked in samples that have already suffered a partial change in this direction. The final darkening may be attributable to drying, although this may not be so in view of the high humidity maintained in the exposure chamber. In any event, the extent of the darkening appears to be conditioned

TABLE III

COLOUR STABILITY OF INTERNAL SURFACE OF PORK AFTER STORAGE FOR 48 WEEKS AT VARIOUS TEMPERATURES AND RELATIVE HUMIDITIES

Storage temperature, °C.	Exposure period	Observed colour (total scatter for all components)	Colour stability (change in total scatter from initial value)
− 6.6	0	56.7	—
	17	57.9	1.2
	89	55.3	−1.4
−12.2	0	51.5	—
	17	56.2	4.7
	89	49.7	−1.8
−17.7	0	54.2	—
	17	57.3	3.1
	89	49.7	−4.5
−29.0	0	54.0	—
	17	56.4	2.4
	89	47.3	−6·7

Analysis of variance
(After 17-hr. exposure)

Variance attributable to	Degrees freedom	Blue	Green	Red	Total
Between temperatures	3	4.44*	3.31	6.38**	19.1
Between humidities	4	0.54	3.05	0.89	10.4
Residual[1] (T × H)	12	0.66	1.27	0.76	6.68
After 89-hr. exposure					
Between temperatures	3	18.2 *	23.9 **	3.93	121 **
Between humidities	4	0.76	2.97	4.93	21.7
Residual[1] (T × H)	12	4.78	2.08	2.15	12.1

**Indicates 5% level of significance.*
***Indicates 1% level of significance.*
[1] *Residual (T × H) significantly exceeds error of duplicates in few instances.*

by the extent of brightening due to methaemoglobin formation during previous storage or exposure. Previous results (5, 7) indicate that initially bright samples of bacon are most likely to suffer darkening during exposure. The opposite effect observed in the present study may be attributed to a difference between pork and bacon, which seldom becomes brighter, or because the increased brightness was due to methaemoglobin as distinct from a lower pigment level.

TABLE IV

PEROXIDE OXYGEN CONTENT OF PORK FAT AFTER STORAGE FOR 48 WEEKS AT VARIOUS TEMPERATURES AND RELATIVE HUMIDITIES

Mean content of peroxide oxygen for all relative humidities at each storage temperature

Temperature, ° C.	− 6.6	−12.2	−17.7	−23.0
Peroxide oxygen*†	19.9	1.45	0.54	0.46

Mean content of peroxide oxygen for all temperatures at each relative humidity

Relative humidity, %	83	87	92	96	100
Peroxide oxygen*†	6.99	5.47	5.05	4.57	5.83

Analysis of variance

Variance attributable to	Degrees freedom	Mean square
Temperature	3	455 ***
Humidity	4	3.38
Residual[1] (T × H)	12	3.36

†*As ml. of 0.002N sodium thiosulphate.*

**Necessary difference, 5% level of significance: 1.97.*

****Indicates 0.1% level of significance.*

[1] *Residual (T × H) did not significantly exceed error of duplicates.*

TABLE V

FREE FATTY ACID CONTENT OF PORK FAT AFTER STORAGE FOR 48 WEEKS AT VARIOUS TEMPERATURES AND RELATIVE HUMIDITIES

Mean content of free fatty acid for all relative humidities at each storage temperature

Temperature, ° C.	−6.6	−12.2	−17.7	−23.0
Free fatty acid†*	1.15	0.67	0.48	0.37

Mean content of free fatty acid for all temperatures at each relative humidity

Relative humidity, %	83	87	92	96	100
Free fatty acid†*	0.67	0.66	0.67	0.67	0.65

Analysis of variance

Variance attributable to	Degrees freedom	Mean square
Temperature	3	1.19 ***
Humidity	4	0.001
Residual[1] (T × H)	12	0.001

†*As % oleic acid.*

**Necessary difference, 5% level of significance: 0.03.*

****Indicates 0.1% level of significance.*

[1] *Residual (T × H) did not significantly exceed error of duplicates.*

Development of Rancidity

Temperature was the primary storage condition affecting the formation of peroxide oxygen in the fat (Table IV). Although an increase in temperature was in all instances associated with an increase in the peroxide oxygen content, the observed differences were statistically significant only for a change from $-12.2°$ to $-6.6°$ C. The mean values for each temperature at all relative humidities show that for periods comparable to that studied here, pork should be stored at $-12.2°$ C. (preferably at $-17.7°$ or $-23°$ C.) if rancidity in the fat is to be avoided. There was some indication that peroxide oxygen formation decreased with increase in relative humidity, but the differences noted were not statistically significant. It is considered that temperature and not the presence of moulds, was the prime factor responsible for the high peroxide oxygen content observed at $-6.6°$ C.

Storage temperature alone had a significant effect on free fatty acid formation (Table V). It may be seen, from the mean values at each temperature over all relative humidities, that successive decreases in temperature were in each instance associated with statistically significant decreases in the free fatty acid content. It is to be noted, however, that even at temperatures as high as $-6.6°$ C., the content of free fatty acid was small, and would contribute little to spoilage in pork fat. Variations in the relative humidity had no apparent effect on free fatty acid formation.

Acknowledgments

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CANADIAN WILTSHIRE BACON

XV. QUANTITATIVE BACTERIOLOGICAL AND CHEMICAL CHANGES IN TANK PICKLE AND ON BACON DURING CURE AND MATURATION[1]

By N. E. Gibbons[2] and W. Harold White[3]

Abstract

Little or no change due to time or depth was found in the bacterial content of Wiltshire tank pickle during cure. A decided increase in the number of bacteria occurred on the surface of the sides. Settling of bacteria from the pickle was not responsible for this increase. Of the normal commercial practices of salting the sides prior to cure, and washing and wiping after cure, wiping was the most important in reducing the bacterial population of the surface of the meat.

The concentration of sodium chloride, nitrate, and nitrite in the curing pickle decreased most rapidly during the first 12 or 24 hr. of cure. Over the remainder of the curing period, the chloride content decreased at a relatively uniform rate and the nitrite content remained constant within the sampling error. Changes in nitrate were generally less than the sampling error. Although stratification of the salts in the tank was evident even after three hours from the beginning of cure, the actual magnitude of the differences was small and would have little detrimental effect on the bacon. There was no apparent relation between bacterial and chemical changes in the pickle during cure.

Introduction

It is generally believed that the presence of bacteria is essential for the conversion of pork to bacon. If so, the conditions prevailing in the curing of Wiltshire sides, namely, the high salt concentration in the curing pickles, the low temperature of the curing cellars (3.3° to 4.5° C.), and the short cure (six to eight days), are favourable for the rapid growth of only halophilic and psychrophilic organisms. From previous studies (4) it appeared that the bacterial count of curing pickles increased slightly during cure, but the data did not indicate whether multiplication was just beginning or whether a rapid development and decline had occurred. The present investigation was undertaken to determine some of the bacteriological and chemical changes taking place during the curing of Wiltshire bacon, and, in addition, to study the effect of certain plant practices, such as salting, washing, and wiping, on bacterial development.

Methods

All the experiments were performed under actual plant conditions. The composition and treatment of the pickles, pumping practice, and cure were those normally employed in the plant. The sides selected for sampling formed

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[3] *Biochemist, Food Storage and Transport Investigations.*

part of the regular cure. Although the curing tanks were only four to five feet deep, it was considered that differences in the bacterial and chemical composition of the cover pickle might exist between the top and bottom of the tank. Provision was therefore made for securing samples of the curing pickle at these two levels.

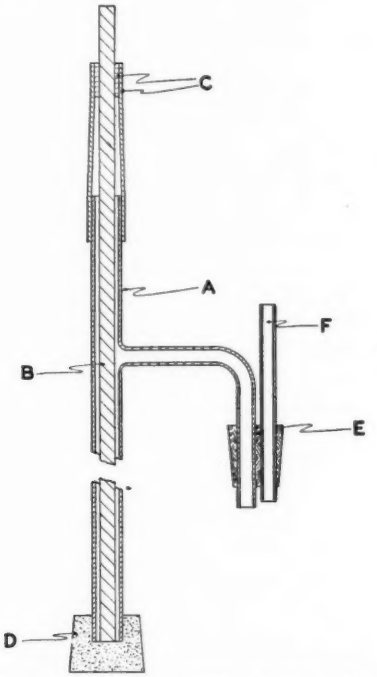

FIG. 1. *Device employed for obtaining samples of curing pickle from curing vats.*

To insure that the samples were drawn from the required depth without contamination, the sampling device shown in Fig. 1 was employed. It consisted of a glass tube, *A*, of suitable length, bearing a side-arm approximately three inches from the top and containing a stout glass rod, *B*, approximately six inches longer than *A*. Rubber tubing, *C*, between the glass tube and rod served to maintain an air tight connection at the top while permitting the displacement of the cork, *D*. In use the cork was pushed off by means of the rod, a sterile flask placed in position at *E* and the pickle sample removed from the desired depth by applying suction at *F*.

In order to obtain samples near the bottom of the tank, tubes, corresponding to the number of samples required, were fastened to a wooden frame, the side-arms were wrapped in paper, and the whole assembly was sterilized in a large sterilizer. The corks, *D*, were then covered with sterile paraffin wax, followed by a layer of Parafilm. Waxed paper was also placed around the wrapped

side-arms. The wooden frame, which was fastened in the curing tank, allowed sides to be packed closely around it and at the same time insured that all samples were taken from the same depth. Top samples were removed by lowering single unmounted tubes to the desired depth.

The media used and the general methods employed for the bacteriological study of pickles and bacon have been described (4, 5). Plates were incubated at 20° C., unless otherwise stated. For bacteriological examination of the meat, 12 sq. cm. of the pleural membrane, comprising 3 sq. cm. from each of the third, sixth, ninth, and twelfth ribs, were taken at each sampling. Physiological saline (the salt concentration to which the organisms would normally be subjected) was used to dilute meat samples taken from sides before cure. However, a 4% salt solution was used for the studies on cured meat since it has been shown that at this concentration there was the greatest development of the organisms found on bacon (5).

In one instance an attempt was made to assess the number of bacteria present in curing pickle which were capable of growing under anaerobic conditions. Using the same dilutions and media as for aerobic counts, plates were poured and incubated at room temperature (24° C.) under anaerobic conditions obtained by the method of Weiss and Spaulding (6). Nutrient agar plates were counted after 12 to 14 days, and 4 and 10% salt agar plates after 18 to 21 days.

The methods employed for the determination of the concentration of chloride nitrate, and nitrite in bacon and curing pickle have been given (7).

Experimental Procedure

Two samples of the pump pickle were taken approximately one hour apart from the pump needle. The cover pickle was sampled as it entered the tank, first when the tank was about half-filled and again when nearly filled. During cure the cover pickle was sampled for chemical analysis 2, 5, 9, and 14 hr. after the tank had been filled and daily thereafter. Samples for bacteriological analysis were taken two hours after filling and then after one, three, five, and six days. Two sets of samples were obtained on each occasion from sampling tubes located about two feet from each wall and in diagonally opposite corners of the 14 by 15 ft. curing tank. Samples were obtained from two depths at each position, namely, 12 and 42 in. below the surface, the latter being about 10 inches from the bottom. After curing for six days, the cover pickle was drained from the tank, the sides were allowed to drain overnight, and then refloated with the mixed Wiltshire pickle from the storage tank. This was the only departure from the regular routine, as the sides are not usually allowed to drain in the tank.

Four sides were sampled for surface bacterial content about four hours before placing in the tank. These, together with two unpumped sides, were placed midway up the stack of sides near one of the positions from which the pickle samples were taken. They were not resampled until after removal

from the tank. After draining for one day in the draining cellar at 4.4° C., the sides were baled without wiping and stored at 1.1° C. for 16 days, i.e., 24 days from the beginning of cure. The ribs and gammons were sampled after removal from cure and after storage for bacteriological and chemical analysis.

Changes During Cure

Curing Pickle

The results of the chemical analysis appear in graphical form in Fig. 2. The two curves appearing for each constituent represent the decrease in composition at the two depths, averaged for both horizontal positions in the tank. The cross hatched section represents the decrease in concentration necessary to exceed the 5% level of statistical significance, as computed from the error of sampling the tank, i.e., between positions. The magnitude of the differences in concentration of the salts between the top and bottom of this shallow tank was quite small, and the stratification would have little detrimental effect on the quality of the bacon.

The most rapid changes in concentration of all three curing salts occurred during the first 24 hr. of cure. Over the rest of the curing period the chloride

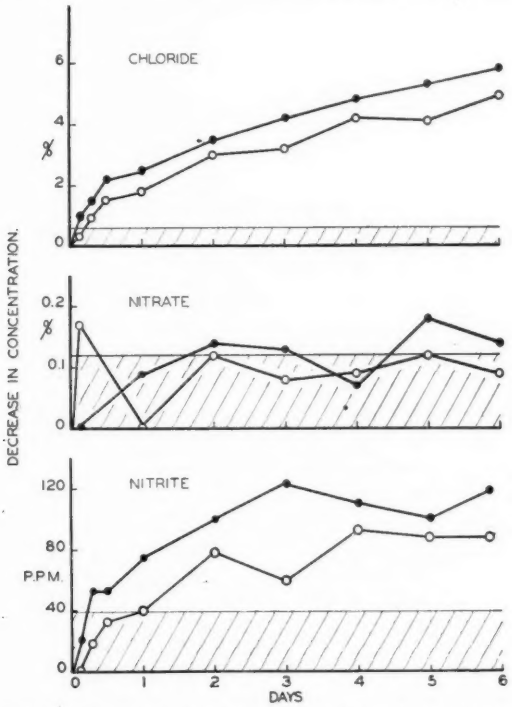

FIG. 2. *Decrease in the concentration of sodium chloride, nitrate, and nitrite in Wiltshire tank pickle during cure.* ● *indicates values for top position in the curing tank,* ○ *for bottom position. The cross hatched section represents the necessary difference from zero decrease.*

content decreased at a relatively uniform rate, whereas the concentration of nitrite remained practically constant. It is reasonable to assume that the diffusion of nitrite into the tissue continued throughout cure. This, together with the destruction of nitrite by nitrite reducing bacteria, appears to balance approximately its formation by nitrate reducing bacteria. Although the nitrate content decreased slightly, the observed changes were usually not significantly greater than the sampling error. For the most part, the changes in the concentration of the three salts at the two depths studied were comparable.

TABLE I

ANALYSIS OF VARIANCE FOR CHANGES IN SODIUM CHLORIDE, NITRATE, AND NITRITE CONTENT OF WILTSHIRE TANK CURING PICKLE DURING CURE

Variance attributable to	Chloride		Nitrate		Nitrite	
	D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Time	9	26.8**	7	0.0189*	9	11,800**
Depth	1	10.0**	1	.0023	1	13,700**
Sampling error	20	0.15**	16	.0066**	20	700
Residual	49	.03	39	.0002	49	500

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

Statistical analyses were made to determine the significance of the differences attributable to the various factors studied. The variance could be analysed into portions attributable to the effect of time, depth, position, the differential effects of these factors, and the analytical error derived from duplicate tests in the laboratory. Although the differential effects were usually significantly greater than the residual variance, they did not differ significantly within themselves or from the variance between horizontal positions. Consequently the estimate of sampling error used for statistical comparison here was obtained by grouping the variance due to horizontal position in the tank with the first order interactions between the various factors studied. The results of the analysis showed that, for chloride and nitrite, the effect of both time and depth were significantly greater than the sampling error; for nitrate (Table I), time alone had a significant effect.

Bacteriological analysis showed little difference between two samples of pump pickle taken from the pump needle one hour apart. Average logarithmic counts of 5.84 and 5.26, were obtained on 10 and 4% salt agar and of 4.58 and 3.90 on nutrient agar at 20° and 37° C., respectively.

The samples of cover pickle taken as it was flowing into the tank were practically identical in bacterial content with those removed from the tank. The average logarithmic count was 6.95 and 6.33, on 10 and 4% salt agar and 5.17 and 4.13 on nutrient agar at 20° and 37° C., respectively.

The results of the bacteriological analyses during cure are presented in Fig. 3 and Table II in the same form as the chemical data. From the graph (Fig. 3, Tank 1), in which the logarithm of the average number of organisms found in the two positions is plotted against time for each of the four media, it is evident that the changes are small, and in some instances, somewhat irregular.

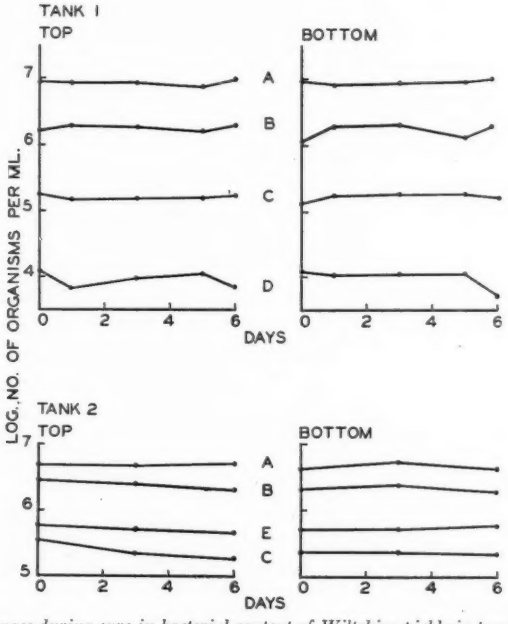

FIG. 3. *Changes during cure in bacterial content of Wiltshire pickle in two tanks (1 and 2) as estimated on (A) 10% salt agar at 20° C., (B) 4% salt agar at 20° C., (C) nutrient agar at 20° C., (D) nutrient agar at 37° C., and (E) 20% salt agar at 20° C.*

Statistical analysis of the logarithms of the number of organisms found on four combinations of media and incubation temperatures indicate that the effect of time alone was significant. Moreover, this was so only for those organisms capable of growth on nutrient agar at 37° C. and on 4% salt agar at 20° C. Since the significant changes were irregular, an interpretation of their nature is impossible.

The bacteriological changes during cure in a later experiment (page 70) are also shown in Fig. 3 (Tank 2). In this instance a medium containing 20% salt was used but only about one-tenth as many organisms developed as on 10% salt agar. On no medium was an increase in the number of organisms found during cure.

The results presented here failed to confirm a previous observation that spent pickle contained three to four times as many organisms as cover pickle (4). In the previous investigation the majority of the pickle samples were

TABLE II

ANALYSIS OF VARIANCE FOR CHANGES IN BACTERIAL CONTENT OF WILTSHIRE TANK PICKLE DURING CURE

Variance attributable to	D.f.	Mean square			
		Dist. water nut. agar 37° C.	Dist. water nut. agar 20° C.	4% brine 4% salt agar 20° C.	10% brine 10% salt agar 20° C.
Time	4	0.193**	0.00919	0.0739*	0.0163
Depth	1	.028	.00226	.0029	.0287
Sampling error	13	.031**	.00472**	.0145**	.0064**
Residual	40	.004	.00045	.0011†	.0005

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*
† *38 degrees of freedom.*

obtained by dipping from the surface. It was therefore considered possible that differences might exist in the bacterial content of surface and subsurface layers of the curing pickle. To test this supposition, samples were taken at the surface by dipping with a flask and from just below the surface by the sampling device described earlier. In addition, samples were taken prior to the entry of the pickle into the tank and upon the removal of the sides.

The results (Table III) show that most of the surface samples contained slightly more organisms than those from the subsurface. However, the difference would account for only a small proportion of that found previously. Since the bacterial content of the pickle was greater after the sides had been removed than before the tank was disturbed, it is possible that the washing of organisms from the sides may account in part for increases observed in the population of the pickle. However, since this pickle was pumped off and

TABLE III

EFFECT OF SAMPLING POSITION ON THE NUMBER OF ORGANISMS IN WILTSHIRE TANK PICKLE. LOGARITHM OF THE NUMBER OF ORGANISMS PER MILLILITRE

Sample	Nut. agar		10% salt agar	
	Days		Days	
	0	8	0	8
1	5.21[1]	5.66[2]	6.24[1]	6.44[2]
2A	5.17	4.96	6.12	6.01
2B	5.27	5.09	6.20	6.09
3A	5.19	4.90	6.21	6.06
3B	5.25	5.00	6.21	6.10

[1] *Taken from brine line.*
[2] *Taken from tank after sides removed; mixed with other pickle.*
A. *Samples from 6 in. below the surface.*
B. *Samples dipped up from surface in flask.*

mixed with other pickle prior to floating the sides, the increase might be due to the added pickle. It is of interest to note that in this particular cure the bacterial content of the spent pickle was in all instances lower than that of the cover pickle.

The results obtained for the number of organisms in curing pickle which were capable of anaerobic growth (Table IV) indicated a slight decrease in number during the early part of cure, followed by an increase. The depth in the tank at which the sample was taken had little apparent effect. Although no attempt has been made to classify the types of organisms present, the majority were capable of aerobic growth and were therefore facultative anaerobes.

TABLE IV

THE NUMBER OF ORGANISMS CAPABLE OF ANAEROBIC GROWTH IN PUMP PICKLE AND IN TANK PICKLE DURING CURE, EXPRESSED AS LOGARITHM OF THE NUMBER OF BACTERIA PER MILLILITRE

Medium	Top			Bottom			Pump pickle	
	Days						A	B
	0	3	6	0	3	6		
Position 1								
Nut. agar 24° C.	3.62	3.31	3.99	3.49	3.28	3.90	3.27	3.11
4% salt agar	5.34	5.11	5.91	5.37	4.04	5.71	4.68	4.36
10% salt agar	5.77	5.31	6.18	5.87	4.40	6.02	4.75	4.50
Position 2								
Nut. agar 24° C.	—	3.00	3.95	3.82	3.16	3.81		
4% salt agar	5.58	4.88	4.90	5.48	4.90	5.83		
10% salt agar	6.10	5.58	6.04	6.06	5.10	6.06		

In the anaerobic chambers the methylene blue indicator usually decolorized overnight in the desiccators containing nutrient agar plates but, in jars containing salt agar plates, the decolorization was extremely slow and at times proceeded only to a green or pale green shade. Furthermore, the rate of decolorization decreased with increased salt concentration of the medium and with the proportion of salt agar plates in the desiccator. No improvement was obtained by evacuating again and filling the desiccators with hydrogen. Since the indicator was decolorized in jars containing no salt agar and slowly in those containing salt agar, it was assumed that anaerobic conditions prevailed in all. It has been found since that this difficulty may be avoided by decolorizing the indicator by heating before placing in the jars. The indicator then remains colourless until the jar is opened.

Bacon

During cure, a decided increase in the number of organisms on the sides was noted. The increase of those organisms capable of growth on 4 and 10% salt agar (Fig. 4) was especially high—100 to 200 times as compared with a maximum increase of 1.7 times in the tank curing pickle. During the storage of the sides after cure, the greatest increase was on 4% salt agar, the salt

concentration most closely approaching that of the bacon. However, in no instance was the rate of increase as great as during cure. This is due, in part, to the lower temperature of storage.

It was considered that the rapid increase in bacterial count on the rib surface of the sides during cure might be due to the settling of bacteria from the pickle. To test this possibility three sides (1 to 3) were placed in cure with the rib surface facing up as customary, and three (4 to 6) with the rib surface down. The results obtained (Table V) for bacterial counts on the rib surface before and after cure (eight days) showed there was a similar increase in the number of organisms regardless of the position of the rib

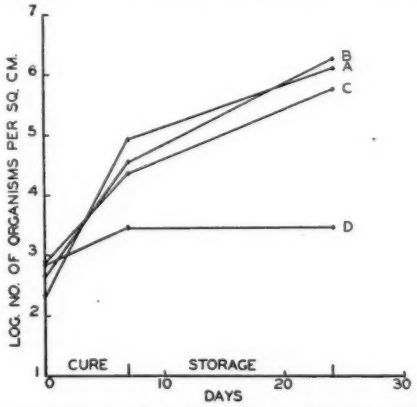

FIG. 4. *Changes in the number of bacteria on the surface of Wiltshire bacon sides during cure (0 to 7 days) and storage (7 to 24 days) as estimated on (A) 10% salt agar, (B) 4% salt agar and (C) nutrient agar at 20° C., and on (D) nutrient agar at 37° C. Averages of four sides.*

TABLE V

EFFECT OF POSITION OF RIB SURFACE OF WILTSHIRE BACON SIDES IN THE TANK DURING CURE ON THE NUMBER OF ORGANISMS PER SQUARE CENTIMETRE EXPRESSED AS THE LOGARITHM

Side	Before cure			After cure		
	Nut. agar	4% salt agar	10% salt agar	Nut. agar	4% salt agar	10% salt agar
1	3.34	3.48	2.90	4.32	4.58	4.64
2	3.62	3.54	2.90	4.71	5.18	5.33
3	4.45	4.22	3.66	4.56	5.79	5.97
4	3.63	3.43	3.00	4.11	4.37	4.56
5	2.79	2.63	2.43	5.91	—	5.83
6	2.86	2.79	2.64	4.45	4.64	4.83
1 Ham	4.43	4.41	4.25	5.15	5.25	5.32
2 Ham	4.43	4.43	4.23	6.15	6.26	5.55
3 Ham	4.85	4.77	4.87	5.76	6.19	5.53

Sides 1 to 3 cured with rib surface up, 4 to 6 with rib surface down.

surface in the curing tank. Samples removed from the exposed lean surface of the gammons both at the beginning and end of cure were usually higher in bacterial content than the ribs. During the cure the rate of increase was essentially the same on both rib and gammon surfaces. It is therefore concluded that factors other than the settling of bacteria from the pickle are responsible for the rapid increase during cure.

The data obtained for the concentration of the curing salts and the distribution of sodium chloride in the hams of pumped and unpumped sides have been given previously (8).

Effect of Washing, Wiping, and Salting on the Surface Bacterial Content of Bacon

In view of the increase in the number of bacteria on the sides during cure, the effect of three common plant practices, namely, salting the sides in the tank, washing, and wiping them after cure, was investigated. For this purpose twelve sides, sampled as previously described for the bacterial content on the rib surface, were divided into three groups of four each. When placed in one corner of the curing tank one group was sprinkled with the usual quantity of salt (16 oz. per side), another with approximately twice this amount, and the third with none. The heavily salted sides were placed nearest the bottom of the tank. Two hours after the beginning of cure and again after three and six days, pickle samples were removed from two positions just below the surface and from one approximately 10 in. from the bottom of the tank.

At the end of six days the sides were removed from cure, sampled, and divided into four groups of three each in such a manner that each group contained a representative of the three salt treatments. After the rib surfaces of two such groups were washed with fresh pickle from a hose, all the sides were drained overnight. On the following day one group of washed and one of unwashed sides were baled without further treatment, whereas the other two groups were, prior to baling, wiped with the customary white burlap cloths which had been washed in hot water at 93° C. (200° F.) and

TABLE VI

EFFECT OF DIFFERENT AMOUNTS OF SALT ON SIDES DURING CURE ON THE INCREASE IN THE NUMBER OF BACTERIA ON THE SURFACE. EXPRESSED AS LOGARITHM OF THE NUMBER OF ORGANISMS PER SQUARE CENTIMETRE, AVERAGE OF FOUR SIDES

	Before cure			After cure		
	Nut. agar	4% salt agar	10% salt agar	Nut. agar	4% salt agar	10% salt agar
Heavily salted	3.72	3.86	3.45	4.56	4.76	4.62
Regular salt	2.64	2.73	2.54	4.69	5.05	4.67
No salt	3.05	3.04	2.87	4.90	5.35	5.16

rinsed in pickle at 71° C. (160° F.) before use. In this manner a similar number of washed, washed and wiped, wiped, and untreated sides were obtained for comparison. After baling, the sides were stored at 1.1° C. for 12 days, a period comparable to shipment to England. After each of the above treatments, samples for bacteriological analysis were removed from the rib surface of each side.

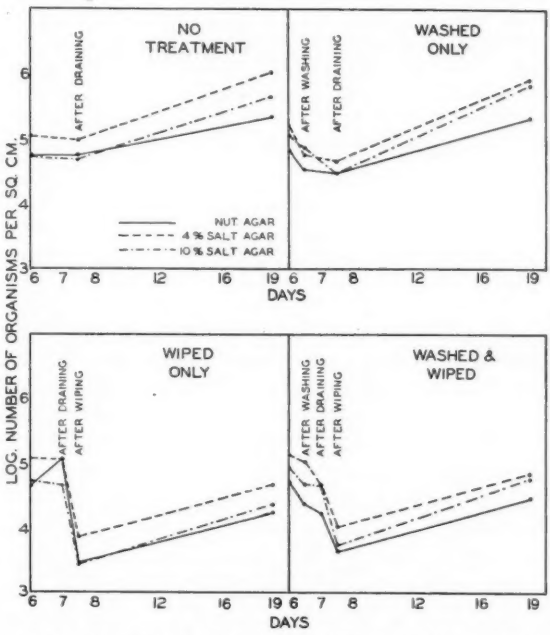

FIG. 5. *Effect of washing, wiping, draining, and storage on the number of bacteria on the surface of Wiltshire sides. Time in days from the beginning of cure. Averages of three sides.*

During cure the number of organisms on the sides increased in all instances (Table VI). The rate was greatest for the sides upon which no salt had been sprinkled and decreased with increasing amounts of salt. Although a random allocation of sides was made for the three groups, all those receiving the heavy salting had significantly higher initial counts than the remainder. Thus, although the final counts for these sides were approximately the same as the others, there is some indication from the growth rates that heavy salting inhibits surface bacterial growth on sides during cure.

The logarithms of the number of organisms per square centimetre of rib surface after the various treatments are shown graphically in Fig. 5. It is apparent that draining had little effect on the bacterial population. Washing caused a slight reduction in the number of organisms. Wiping, however, reduced the count to one-quarter to one-tenth of the original number.

The significance of the differences noted for these various operations was tested statistically. Since the number of bacteria on the sides at the end of cure did not differ significantly, direct comparisons of subsequent procedures can be made. A t test on the differences in the logarithm of the number of organisms before and after draining gave insignificant values (t being 0.33, 0.76, and 0.65 for nutrient agar, 4, and 10% salt agar, respectively).

TABLE VII

ANALYSIS OF VARIANCE OF DIFFERENCES IN LOGARITHM OF THE SURFACE COUNTS OF 12 SIDES OF WILTSHIRE BACON BEFORE AND AFTER TREATMENT

Variance attributable to	D.f.	Mean square		
		Nut. agar	4% salt agar	10% salt agar
Salting	2	1.51	2.25	2.06
Washing, etc.	3	9.91*	7.12	9.54*
Error	6	1.41	1.72	1.03

* *Indicates 5% level of significance.*

An analysis of variance of the differences in the logarithm of the counts before and after the different operations showed that the amount of salt sprinkled on the sides during cure had no significant effect on later operations (Table VII). The other treatments, i.e., washing and wiping, had a significant effect on the number of organisms growing on nutrient agar and 10% salt agar.

Statistical methods were employed to determine whether washing or wiping had the greater effect. Table VIII shows that wiping, but not washing, had a significant effect on the number of organisms growing on all media.

Since the lower number of organisms on the sides after wiping was reflected in a proportionately lower number after storage (Fig. 5), it may be assumed that the growth rate during the storage period was approximately the same on wiped and unwiped sides. Wiping, as practised in this investigation, should therefore lengthen the slime-free life of the sides (5). An analysis of variance of the logarithm of the total number of organisms on the sides at

TABLE VIII

ANALYSIS OF VARIANCE OF TOTAL DIFFERENCES IN LOGARITHM OF SURFACE COUNT OF WILTSHIRE SIDES TO SHOW EFFECT OF TREATMENTS, OTHER THAN SALTING, DURING CURE

Variance attributable to	D.f.	Mean square		
		Nut. agar	4% salt agar	10% salt agar
Washing	1	0.47	0.71	0.49
Wiping	1	28.8 **	19.8 *	26.5 **
Interaction	1	0.47	0.90	1.65
Error	8	1.44	1.85	1.29

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

the end of storage showed that wiping was the only treatment that had a significant effect on the final number of bacteria present (Table IX). Neither washing nor salting as carried out in this investigation had a beneficial action.

TABLE IX

ANALYSIS OF VARIANCE OF LOGARITHM OF TOTAL NUMBER OF ORGANISMS ON SURFACE OF WILTSHIRE SIDES AFTER STORAGE

Variance attributable to	D.f.	Mean square		
		Nut. agar	4% salt agar	10% salt agar
Salting	2	1.28	0.91	0.95
Wiped—not wiped	1	21.8 *	31.4 *	34.8 *
Other treatment	2	0.68	0.51	0.43
Error	6	3.36	5.23	3.11

* *Indicates 5% level of significance.*

Discussion

Previous work suggested that the number of bacteria increased in the pickle during the cure of Wiltshire sides (4). The results of the present investigation indicate that in the plant in which the tests were made such changes are small, if they occur at all. This is not considered to be a discrepancy, but attributable to the differences in sampling and the method of handling the samples in the plant and under the carefully controlled conditions of these studies. Since there was a considerable increase in the number of organisms on the sides, it is concluded that any changes attributable to bacterial action in the conversion of pork to bacon occur at the meat-pickle interface.

In a recent report of a few preliminary experiments (1), it was found that the number of organisms in pickle increased during cure, and that a reduction or very slight increase occurred on sides. However, since in those experiments the bacterial content was purposely reduced to a minimum, the results are not strictly comparable with those on commercial cures presented here.

Although no statistical computations were made, there is apparently little relation between the changes in bacterial and nitrate or nitrite content of the pickle during cure. This does not preclude the existence of such a relation, however, since the formation of nitrite from nitrate as a result of bacterial action may be masked by its reaction and diffusion into the tissue, or its reduction to other nitrogen compounds.

Acknowledgments

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INTERACTION BETWEEN THE AUTOSOMES OF *DROSOPHILA MELANOGASTER* AS MEASURED BY VIABILITY AND RATE OF DEVELOPMENT[1]

By S. C. Reed[2]

Abstract

Females of an inbred *al c : se ss : ey*^{R} mutant strain were crossed with males from a "wild" strain that had been inbred (brother × sister) for more than 60 generations. The F_1 males were mated with mutant strain females. The backcross offspring would be expected to appear in eight genotypes with equal frequencies were there no differences in viability. The marker genes permitted the scoring of each fly as to whether it was heterozygous or homozygous for each of the mutant autosomes.

The genes used as markers probably had a distinctly negative effect upon both viability and rate of development, but the second pair of autosomes in combination with the other mutant autosomes increased viability, when homozygous, to a greater extent than did a heterozygous mutant-wild type pair. Apparently the possible negative effects of the markers *al* and *c* were more than offset by other genes on this autosome which had positive effects. The negative effects of the homozygous mutant third and fourth chromosomes were severe when in combination.

The interactions of the positive second chromosome and the negative third and fourth chromosomes in the eight genotypes were of some geometric order. It was found that the addition of a "positive" or "negative" autosome to any genotype caused a change in viability in the direction of the added autosome, but the *amount* of change is at present unpredictable and depends upon the particular combination to which the autosome was added.

The relation between the different autosomes and the rate of development was quite different from the relation between autosomes and viability. The second chromosome (marked by *al c*), which gave the only positive contribution to viability, retarded development more than either the third *(se ss)* or the fourth *(ey*^{R}*)*. The effects of the three autosomes on rate of development were not strictly additive.

It is concluded that there is interaction of the genes for quantitative characters and this interaction is geometric in nature. The significance of the results in their relation to some theories of the inheritance of quantitative characters is discussed.

Most of the investigators interested in the inheritance of quantitative characters have chosen to study the inheritance of size. East and Castle were pioneers in the study of size inheritance, but Sax (6) was the first to present a good demonstration of an association between qualitative and quantitative factors. Later MacArthur and Butler (3) provided clear evidence that genes for size of tomato fruits react in a geometric fashion. Charles and Smith (1) came to the same conclusion regarding gene interaction in tomatoes and tobacco and published useful algebraic formulae that enable one to distinguish between arithmetic and geometric gene action from a study of the means, variances, and third moments of the F_2 and other generations.

[1] *Manuscript received September 9, 1940.*

Contribution from the Department of Genetics, McGill University, Montreal, Que. Research supported by a grant for genetics and cytology from the Rockefeller Foundation to McGill University.

[2] *Now at the Biological Laboratories, Harvard University, Cambridge, Mass.*

Rasmusson's (5) theory of genic interaction assumes that the total effect of the genotype is not determined by the direct simple addition of the effects of quantitative genes, but that they interact with one another in such a way that the cumulative effect of all the genes is less than the sum of their individual effects; the distributions should be negatively skewed according to this conception. Powers (4) in his work on barley obtained data that support the view that the cumulative effect of a number of genes is greater than the sum of their individual effects. This hypothesis calls for positive skewness of the distributions. Sinnott (7) found, in experiments with *Cucurbita*, that 41 out of 43 F_2 populations had definite positive skewness. An F_2 distribution that forms a normal curve is assumed to show the absence of gene interaction.

These views of the relation between curve shape and type of factor interaction are rather an over-simplification because, as Charles and Smith (1) have shown, positive skewness is an indication of geometric factor effects only if large and small parent strains have equal non-genetic variances. If the larger strain has a larger standard deviation, as is perhaps more commonly the case, some positive skewness is thereby introduced into the F_2, whatever the type of factor interaction.

Because *Drosophila* is so valuable for the study of qualitative characters and such poor material for the study of size inheritance remarkably little work has been done on quantitative inheritance in this species. However, many characters of a quantitative nature, other than size, could be studied profitably in *Drosophila*. Timofeeff-Ressovsky (8) was one of the many investigators aware of the great advantages that *Drosophila* presents for a study of some quantitative characters and accordingly studied the effect on vitality of six sex-linked genes in *Drosophila funebris*. It was found that the gene "eversae" increased vitality relative to the normal allele, but that the other five mutant genes decreased vitality. The crosses were between isogenic strains and allowed exact calculation of the effects and interactions of each of the six mutations. Timofeeff-Ressovsky found large deviations of the observed from the expected values on a strictly additive basis. Thus there were interactions between the genes but the interactions followed no uniform rule. Each combination of genes produced a new and unrelated vitality threshold. The work with *Drosophila funebris* was concerned with the effects of six mutations all located on the *X* chromosome. The material to be presented here concerns the effects of the three autosomes of *Drosophila melanogaster*. Whereas Timofeeff-Ressovsky demonstrated the type of reactions that may be expected from mutant genes all of which are within the same chromosome, the present work gives a clear description of the interactions between whole chromosomes. The types of interactions for two distinct quantitative characters, viability and rate of development, will be demonstrated. The absence of crossing-over between any of the chromosomes involved in the generations studied allows an analysis of chromosome interactions which would not be feasible with plant and most animal material. In this paper the term "viability" has been used rather than Timofeeff-

Ressovsky's "vitality". The meaning of both terms is the same here, i.e., the production of an adult fly from the fertilized egg. The duration of life has not been considered in these experiments; data concerned with that problem were published by Gonzalez (2).

Methods

Females of an inbred strain that bore markers on the three pairs of autosomes were crossed with males of a "wild" type strain that had been inbred for more than 60 generations. The F_1 males were of the "wild" phenotype but were heterozygous for each of the autosomes from the mutant strain mother. This mutant strain was generally known as the "C" stock and possessed the marker genes, aristaless *(al)* and curved *(c)* on the second chromosome. The third chromosome of the mutant race was marked with the genes sepia *(se)* and spineless *(ss)*, and the fourth chromosome carried the marker gene eyeless *(ey^R)*. The F_1 males, in which, of course, there was no crossing-over, were mated with females of the mutant "C" stock to produce a backcross generation. The backcross progeny all derived their sex chromosomes from the "C" stock but were homozygous or heterozygous for the "C" stock genes on the autosomes. The marker genes allow scoring the backcross flies for the racial origin of each autosome that had the precise genic composition of the race from which it was originally derived.

Eggs from the mating of the pure "C" stock females with the F_1 males were deposited in large numbers upon corn meal agar in Petri dishes. The eggs were removed from the Petri dishes a few hours after they were laid and placed in large vials, 20 eggs in each vial. The vials contained "slants" of corn meal agar lightly sprayed with yeast one day before use. The flies appearing in each vial were scored for sex and autosomal constitution within 12 hr. after emergence from the pupa case. If all classes were equally viable, they should fall into eight different genetic classes with equal frequencies or, with the sexes treated separately, into sixteen classes.

Crude methods were used in studying the differences in the rates of development of the different genotypes. A very accurate method involving continuous observation of each egg was developed but, as the number of eggs that could be followed at one time was small, the crude method, which allowed observations in large numbers, was more valuable for this particular problem. The crude method consisted simply of recording the order in which flies of the various genotypes emerged from their pupa cases. If it were impossible to tell which fly in a vial was the first or the last to emerge, that vial was discarded in the study of the rate of development. It is realized that the eggs when laid were not all in the same stage of development.

The eggs and flies were always kept in an incubator held at $24° \pm 0.5°$ C. The incubator was large enough to accommodate the investigator. All observations were made by the author himself and this should have tended to reduce experimental error.

Examination of the salivary chromosomes of the strains showed them to be apparently normal. Certainly they had no large inversions or deficiencies.

Experimental Data

Viability

The viability of the parental strains and backcross flies is shown in Table I. The difference in viability between the wild inbred stock and the mutant inbred stock is very great, amounting to 54.4%. The viability of the F_1 male genotype (in "C" cytoplasm) and of the whole backcross generation is approximately intermediate between that of the pure strains, though nearer that of the "C" stock. It is clear that though all the marker genes were completely recessive with regard to visible characters, the effects of the whole autosomes on viability of the F_1 gave intermediacy or less. The genes concerned with the difference in viability between the two strains collectively, but not necessarily individually, show lack of dominance such as is often associated with quantitative characters.

TABLE I

VIABILITY OF PARENTAL STRAIN, OF F_1 MALES, AND THE BACKCROSS GENERATION AS A WHOLE

Strain	Number of eggs	Number of flies emerged			Viability, %
		♀♀	♂♂	Total	
Wild	2000	735	751	1486	74.1
F_1	1130	230	214	444	39.3
Backcross to "C" stock	5500	1124	1165	2289	41.6
Mutant "C"	1500	122	174	296	19.7

The results for each genotype of the backcross generation are shown in Table II. It will be seen that only one of the markers on each of the second and third chromosomes is listed in the genotype. This is merely a convenient abbreviation; the other markers were also present, of course.

There is clearly a differential viability of the different genotypes but no statistically significant difference between the viability of the wild type heterozygote $\left\{\frac{+}{c}\ \frac{+}{se}\ \frac{+}{ey}\right\}$ and the flies homozygous for the second autosome $\left\{\frac{c}{c}\ \frac{+}{se}\ \frac{+}{ey}\right\}$ or those homozygous for the third autosome $\left\{\frac{+}{c}\ \frac{se}{se}\ \frac{+}{ey}\right\}$. The decrease in viability when the *ey* gene is homozygous $\left\{\frac{+}{c}\ \frac{+}{se}\ \frac{ey}{ey}\right\}$ is highly significant. It is very probable that the decrease in viability may be attributed to the action of the *ey* gene itself, particularly as *ey* disturbs the morphology of the head so markedly.

The sepia eyeless flies with the genotype $\left\{\frac{+}{c}\ \frac{se}{se}\ \frac{ey}{ey}\right\}$ have the lowest viability. Their viability is significantly lower than that of the class of flies

that is genetically identical with the homozygous "C" stock $\left\{\frac{c}{c}\ \frac{se}{se}\ \frac{ey}{ey}\right\}$. This is perhaps surprising because segregates homozygous for the three mutant autosomes might be expected to have a lower viability than segregates homozygous for only two mutant autosomes. An explanation for this appears when it is noted that homozygosity for the autosomes marked by the "curved" gene raised viability in two different combinations of the mutants (Table III). Considering all the combinations, it is found that the "curved" chromosome increased viability in two combinations, left it unchanged in one, and decreased it in the fourth; the third chromosome decreased viability in three combinations and left it unchanged in one, whereas the fourth decreased viability in all four combinations. The increase in viability resulting from the addition of the homozygous "curved" chromosome is highly significant

TABLE II

RELATIVE VIABILITY OF THE DIFFERENT GENOTYPES OF THE BACKCROSS GENERATION. OF THE TOTAL OF 5500 EGGS STARTED ABOUT 688 SHOULD BE EXPECTED TO BE OF EACH OF THE EIGHT GENOTYPES*

Genotype	First experiment	Second experiment	Third experiment	Totals	Viability of the different genotypes relative to $\frac{+}{c}\ \frac{se}{se}\ \frac{+}{ey}$, %
$\frac{+}{c}\ \frac{+}{se}\ \frac{+}{ey}$	72	66	221	359	95.5
$\frac{+}{c}\ \frac{se}{se}\ \frac{+}{ey}$	76	62	238	376	100.0
$\frac{c}{c}\ \frac{+}{se}\ \frac{+}{ey}$	75	74	205	354	94.2
$\frac{+}{c}\ \frac{+}{se}\ \frac{ey}{ey}$	55	30	163	248	66.0
$\frac{c}{c}\ \frac{+}{se}\ \frac{ey}{ey}$	63	66	203	332	88.3
$\frac{c}{c}\ \frac{se}{se}\ \frac{+}{ey}$	53	45	195	293	77.9
$\frac{+}{c}\ \frac{se}{se}\ \frac{ey}{ey}$	24	13	80	117	31.2
$\frac{c}{c}\ \frac{se}{se}\ \frac{ey}{ey}$	40	34	136	210	55.8
	458	390	1441	2289	

**The sexes have been treated separately but as there were no significant differences between them the data are not included here.*

TABLE III

THE MODIFICATION OF VIABILITY RESULTING FROM VARIOUS COMBINATIONS OF THE HOMOZYGOUS MUTANT AUTOSOMES. IN COMBINATION THE CURVED $\left(\frac{c}{c}\right)$ AUTOSOME ALWAYS INCREASES VIABILITY BUT SEPIA AND EYELESS AUTOSOMES ALWAYS DECREASE VIABILITY.

Mutant autosome combination	Effect on viability, %	Mutant autosome combination	Effect on viability, %	Mutant autosome combination	Effect on viability, %
$\frac{c}{c}$ with normal	Unchanged	$\frac{se}{se}$ with normal	Unchanged	$\frac{ey}{ey}$ with normal	Decreased 34.0
$\frac{c}{c}$ with $\frac{ey}{ey}$	Increased 33.8	$\frac{se}{se}$ with $\frac{ey}{ey}$	Decreased 52.8	$\frac{ey}{ey}$ with $\frac{se}{se}$	Decreased 68.9
$\frac{c}{c}$ with $\frac{se}{se}\frac{ey}{ey}$	Increased 79.4	$\frac{se}{se}$ with $\frac{c}{c}\frac{ey}{ey}$	Decreased 36.7	$\frac{ey}{ey}$ with $\frac{c}{c}\frac{se}{se}$	Decreased 28.3
$\frac{c}{c}$ with $\frac{se}{se}$	Decreased 22.1	$\frac{se}{se}$ with $\frac{c}{c}$	Decreased 17.2	$\frac{ey}{ey}$ with $\frac{c}{c}$	Decreased 6.2

and must be considered real. The general trend seems to be one in which the "curved" autosome *increases* viability most when in combination with autosomes that otherwise *decrease* it most. Both the "sepia" and "eyeless" autosomes seem to *decrease* viability to the greatest extent when added to combinations that do likewise. In other words, the lower the viability of a combination, the greater is the *positive* effect if the "curved" autosome is added. But the lower the viability of a genotype, the greater is the effect, in a negative direction, that results when another deleterious autosome in the homozygous condition is added to the combination. The increase in effect is clearly not arithmetic but is a geometric or power function of some sort. Apparently the further from the normal a genotype may be, the more sensitive it is to the addition of a given autosome and the greater the effect on the phenotype. The addition of a positive or negative autosome to any genotype will cause a change in viability in the *direction* of the added autosome, but the *amount* of change is at present unpredictable and depends upon the particular combination to which the autosome was added. Furthermore it should not be considered extraordinary for an autosome to give a positive effect in one combination and a negative effect in some other combination.

Rate of Development

It became apparent after the synthesis of the mutant "C" stock that the rate of development of the "C" flies was slower than that of the "wild" stock. By the crude method of observing the length of time from the laying of the eggs to the emergence of the flies, were obtained the observations on length of the developmental period shown in Table IV. The differences between the average lengths of time taken for development are all statistically significant. At 25° C., the mutant "C" stock took about ten days for development in comparison with nine days for the "wild" stock. Development of the backcross generation covered a wider range of time than the "wild" stock, the average period thus being slightly less. The backcross generation

TABLE IV

DURATION OF DEVELOPMENTAL PERIOD OF THE PARENT STRAINS AND OF THE BACKCROSS GENERATION

Strain	Length of developmental period, days								Total number of flies	Average developmental period, days
	7	8	9	10	11	12	13	14		
	Number of flies									
"Wild"	0	76	1213	180	13	4			1486	9.09 ± 0.01
Backcross generation	41	810	1100	263	67	8			2289	8.79 ± 0.02
"C" stock	0	17	52	168	47	5	4	3	296	9.98 ± 0.05

seemed to give a small display of heterosis, as demonstrated by the faster rate of development.

An inspection of the rates of development of the different genotypes of the backcross generation is of considerable value. Table V shows the percentage of flies of each genotype to emerge first and last. The genotype of the first and of the last fly to emerge in each vial was recorded. The data

TABLE V

THE PERCENTAGE OF FLIES OF EACH GENOTYPE TO EMERGE FIRST AND LAST IN EACH VIAL

Genotype	First fly to emerge		Last fly to emerge	
	No. of cases	%	No. of cases	%
$\frac{+}{c}\ \frac{+}{se}\ \frac{+}{ey}$	64	17.8	3	0.8
$\frac{+}{c}\ \frac{+}{se_a}\ \frac{ey}{ey}$	26	10.5	8	3.2
$\frac{+}{c}\ \frac{se}{se}\ \frac{+}{ey}$	35	9.3	17	4.5
$\frac{c}{c}\ \frac{+}{se}\ \frac{+}{ey}$	26	7.4	18	5.1
$\frac{+}{c}\ \frac{se}{se}\ \frac{ey}{ey}$	6	5.1	8	6.8
$\frac{c}{c}\ \frac{+}{se}\ \frac{ey}{ey}$	13	3.9	23	6.9
$\frac{c}{c}\ \frac{se}{se}\ \frac{+}{ey}$	8	2.7	49	16.7
$\frac{c}{c}\ \frac{se}{se}\ \frac{ey}{ey}$	5	2.4	46	21.9

so obtained are presented in the table. If more than one fly had emerged when observations on a particular vial were made, that vial had to be disregarded for this part of the problem. The evidence from the table is very clear cut. The "wild" type flies emerged first, those homozygous for the "eyeless" autosome next, those homozygous for the "sepia" autosome third, and finally, those homozygous for the "curved" autosome. Flies having these combinations of the autosomes emerged in the order expected from the observations on the retardation of development by the single mutant autosomes. Of the group of flies, in which each was the last in its vial to emerge, the genotypes were again in the order that shows the greatest retardation by the "curved" autosome, next by the "sepia" autosome, and least retardation by the "eyeless" autosome. This is interesting, for the effects on development were precisely the opposite of the effects of the same autosomes on viability. Whereas the "eyeless" autosome reduced viability most, it retarded development least; the curved autosome actually increased viability but at the same time retarded development.

From Table V the expected percentages of the combination genotypes according to various arithmetic additive schemes may be calculated and compared with the observed. This was done but the deviations were too great to allow the consideration that the effects of these autosomes on rate of development were strictly arithmetic. The effects on rate of development fitted more closely an arithmetic additive scheme than did the results on viability, but the deviations were too great in either case to allow the acceptance of such an interpretation. As with viability, it is clear that there was interaction of some geometric kind between the autosomes. It may be predicted that the addition of any one of the homozygous mutant autosomes to any combination of the others will retard development but it is not possible to predict the amount of retardation except for each genotype by itself.

It is of some interest to note that whereas the autosome bearing the mutant gene "eyeless" was the only one that, when homozygous, decreased viability without the aid of one of the other mutant autosomes, each of the three mutant autosomes retarded development without the aid of either of the others.

Discussion

As Powers (4) has pointed out, the nature of the interaction of factors affecting quantitative characters is sufficiently variable to require extensive genetic studies involving a large variety of characters and organisms before an hypothesis of much value for prediction purposes can be formulated. Some of the most valuable experiments for such a formulation, for example, the experiment of Timofeeff-Ressovsky of 1935, may be only indirectly related to the subject. Timofeeff-Ressovsky's work was a demonstration of the production by the use of *X*-rays of mutations affecting vitality. The induced mutations had no observable effects other than their influence upon vitality. These small physiological mutations were shown to be hereditary and though most of them decreased vitality, one case was discovered in which vitality

was improved. The vitality mutations were more frequent than other types and the beneficial ones might be preserved now and again through the action of Natural Selection.

Whereas Timofeeff-Ressovsky (8) studied the interactions between specific genes on a single chromosome and found that two genes working together followed no uniform rule, the author studied interactions between all the autosomes of *Drosophila* at once. It was found, as might be expected, that the amount of change produced by an autosome depended upon the rest of the combination and, because this amount varied for each combination, it is clear that interaction was present and that it was not a simple case of addition of constant values.

It was found further that the most aberrant genotypes were affected to the greatest degree by the addition of an autosome having positive or negative effects on viability. That is, the less normal a genotype, the more sensitive it was to the influence of an autosome affecting viability in either a positive or negative direction. This is another type of illustration of Powers' (4) conception that the cumulative effect of a number of genes is *greater* than the sum of their individual effects.

At first glance this would seem a direct contradiction of the formulation by Rasmusson (5) that quantitative characters have a definite upper limit which, because of mechanical or other reasons, cannot be exceeded. In other words, the more closely the limit is approached by the addition of advantageous genes, the smaller is the effect of each gene; therefore the cumulative effect of all of them becomes less than the sum of their individual effects. The hypothesis of Powers' might be called one of "compound interest increments" and that of Rasmusson one of "diminishing returns". However, since each conception is a picture of the same sort of facts read from a different angle, there is no contradiction. If the effects are read in one direction, the diminishing returns effect is obtained, in the other, the compound interest effect. The case of the addition of the "curved" gene may be considered. Its effect on viability is less and less beneficial, the better the viability of the rest of the genotype; this is a case of "diminishing returns". Looked at from the other direction, the effect becomes greater the poorer the viability of the rest of the genotype—a case of "compound interest". In any allelic pair, one member increases viability and the other decreases it, against a given genetic background. Naturally, the structure and genotype of the organism produce a limit at both ends of the scale of these effects.

In general it may be stated that there is an interaction of the genes for quantitative characters and this interaction is geometric in nature. Various schemes were designed to discover the precise nature of the geometric interaction but none of them were entirely satisfactory. It is perhaps best to report this system of geometric interactions without attempting, at present, a practical scheme that would be weakened by the weight of necessary theoretical assumptions.

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